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tgcttalatcooagatgatatntooogtalelogtgaltogigtgaccoglatcoogaffccfolgooaltglooaccaatcactgagcatcloogoocatalc

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(57) Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder with a lifetime incidence of approximately 2 percent. It was recently reported that a PD susceptibility gene is located on the long arm of human chromosome four. The present invention reports the subsequent identification of a mutation in the alpha synuclein gene, which codes for a presynaptic protein thought to be involved in neuronal plasticity. The finding of a specific molecular alteration which is causative for PD will permit the detailed understanding of the pathophysiology of the disorder, which will lead to potential therapeutic interventions, as well as a means for diagnosing individuals having an increased risk of developing the disease.

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## CLONING OF A GENE MUTATION FOR PARKINSON'S DISEASE

#### BACKGROUND OF THE INVENTION

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#### 1. Field of the Invention

Parkinson's disease (PD) is a common neurodegenerative disorder with a lifetime incidence of approximately 2 percent. A pattern of familial aggregation has been documented for the disorder, and it was recently reported that a PD susceptibility gene in a large Italian kindred is located on the long arm of human chromosome 4. We have identified a mutation in the alpha synuclein gene, which codes for a presynaptic protein thought to be involved in neuronal plasticity, in the Italian kindred and in three unrelated families of Greek origin with autosomal dominant inheritance for the PD phenotype. This finding of a specific molecular alteration which is causative for PD will permit the detailed understanding of the pathophysiology of the disorder. In addition, methods of screening nucleic acids for the presence of mutations in the synuclein gene to test for predisposition to Parkinson's Disease are now possible.

#### 2. Technology Background

Parkinson's disease (PD) was first described by James Parkinson in 1817 (1). The clinical manifestations of this

neurodegenerative disorder include resting tremor, muscular rigidity, bradykinesia and postural instability. A relatively specific pathological feature accompanying the neuronal degeneration is the intracytoplasmic inclusion body, known as the Lewy body, which is found in many regions including the substantia nigra, locus ceruleus, nucleus basalis, hypothalamus, cerebral cortex, cranial nerve motor nuclei, and the central and peripheral divisions of the autonomic nervous system (1).

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In many cases a heritable factor predisposes to the development of the clinical syndrome (2). We have recently shown that genetic markers on human chromosome 4q21-q23 segregate with the PD phenotype in a large family of Italian descent (3). The clinical picture of the PD phenotype in the Italian kindred has been well documented to be typical for PD, including Lewy bodies, with the exception of a relatively earlier age of conset of illness at 46 ± 13 years. In this family the penetrance of the gene has been estimated to be 85%, suggesting that a single gene defect is sufficient to determine the PD phenotype.

We now report the identification of a mutation in the alpha synuclein gene that is associated with Parkinson's disease. The mutation, an AlaE3Thr substitution, was found to be linked to the PD phenotype in four independent PD families and absent from 314 control chromosomes, providing strong genetic evidence that this mutation in the human alpha

synuclein gene is causative for the PD phenotype in these families.

The Ala53Thr substitution is localized in a region of the protein whose secondary structure predicts an alpha helical formation, bounded by beta sheets. Substitution of the alanine with threonine is predicted to disrupt the alpha helix and extend the beta sheet structure. Beta pleated sheets are thought to be involved in the self aggregation of proteins which could lead to the formation of amyloid like structures (6).

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This was already tested in the case of NAC35, the 35 amino acid peptide derived from alpha-synuclein that was first isolated from plaques found in patients with Alzheimer's disease (4). NAC35 was shown to self aggregate and form amyloid fibril which shared the 'amyloid' characteristics of insolubility in aqueous solutions and green birefringence under polarized light, subsequent to Congo red staining (6). NAC35 is located in the middle of the alpha synuclein molecule and extends from amino acid 61 to amino acid 95. Residue 53, which is found to be mutated in PD, is outside the NAC35 peptide found in amyloid plaques. However, the true size of the NAC peptide involved in the plaques is not known since the protease used to isolate the peptide from AD tissue cuts at lysine 60 of the alpha synuclein protein. It is therefore possible that amino acid 53 may be part of the NAC peptide found in plaques. In

crosslinking experiments with beta amyloid (Abeta), it was demonstrated (6) that residues 1-56 and 57-97 specifically bind amyloid and that a synthetic peptide consisting of residues 32-57 performed similarly.

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Three members of the synuclein family have been characterized in the rat, with SYN1 exhibiting 95% homology with the human alpha-synuclein protein (7). SYN 1 of the rat is expressed in many regions of the brain, with high levels found in the olfactory bulb and tract, the hippocampus, dentate gyrus, habenula, amygdala and piriform cortex, and with intermediate levels in the granular layer of the cerebellum, substantia nigra, caudate-putamen, and dorsal raphe (7). This pattern of expression coincides with the distribution of the Lewy bodies found in brains of patients with Parkinson's disease. It is also interesting to note that decrease in olfactory sense often accompanies the syndromic features of Parkinson's disease, and in many cases it is proposed that hyposmia is a prodromic sign of the illness (8).

In the zebra-finch the homologue to alpha synuclein, synelfin, is thought to be involved in the process of song learning, suggesting a role for synuclein perhaps in memory and learning (9). In contrast to humans, rats have a threonine at residue 53 of their homologues to the human alpha synuclein gene (Figure 4). Similarly, the zebra-finch synelfin carries a threonine at amino acid 53, whereas both

Bos taurus and Torpedo californica do not (10). There are no reports that suggest the presence of Lewy bodies in the brains of the rat or the zebra finch or a phenotype resembling that of PD. Lack of any phenotype could be explained by a combination of factors, including the following: the relative short life span of rodents may prohibit the observation of a late onset disorder, interaction with other cellular components not present in the rat may be required for the phenotype, absence of a critical environmental trigger in the rodents, or finally a heterozygous status Ala/Thr may be necessary for the production of a phenotype.

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Studies of early onset AD have previously documented that missense mutations can cause an adult onset neurodegenerative disorder. Of the 31 mutations described so far in the loci for presentilin 1 and 2, thirty were missense and one was a splice variant (11). Missense mutations in the prion protein have also been implicated in the amyloid production seen in Gerstmann-Straussler-Scheinker and Creutzfeld-Jakob diseases, both forms of spongiform encephalopathy (12). Studies in these neurodegenerative disorders have pointed to the importance of the physical chemical properties of mutant cellular proteins in initiating and propagating neuronal lesions leading to disease. Similar studies in the synuclein protein family may provide valuable insights into the etiology and pathogenesis of PD.

Similarly with the mutations in the presentlin genes in patients with early onset Alzheimer's disease, the mutation identified in the alpha synuclein gene is unlikely to account for the majority of sporadic and familial cases of PD. However, this mutation may account for a significant proportion of those families with a highly penetrant, early onset autosomal dominant PD phenotype.

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

## 3. Summary of the Invention

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As described herein, we have discovered that particular mutations in the alpha synuclein gene are associated with predisposition to Parkinson's disease. Accordingly, the present invention includes an isolated nucleic acid comprising a mutated synuclein gene. In particular, the isolated nucleic acid of the present invention contains at least one mutation in the alpha synuclein gene at base pair position 209 of Genbank # L08850, which, in particular, is a change from guanine to adenine. However, since other mutations in the alpha synuclein gene may also lead to Parkinson's Disease (PD), other mutations are also included. In addition, it is conceivable that mutations in the related beta (46) (SEQ ID NO 11) and gamma (SEQ ID NOS 12 and 13)

synuclein genes may also lead to PD. Thus, mutated homologues of the alpha synuclein gene are also included in the present invention. Vectors comprising the isolated nucleic acid and host cells comprising such vectors are included as well.

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Knowledge of particular genes that are associated with PD allows for the search for other specific PD mutations. Accordingly, the present invention also includes a method of using a synuclein gene sequence to identify specific PD mutations. Such mutations may occur in an unrelated population or in a family that demonstrates passage of PD within the family tree.

Since knowledge of mutations associated with Parkinson's disease allows the development of genetic screens that test for an individual's chances of being predisposed to the disease, and such tests may be performed by hybridization analysis using oligonucleotides complementary to the sequence of interest or by PCR amplification using oligonucleotides that are complementary to sequences flanking the mutation, the present invention also includes oligonucleotides complementary to a portion of the synuclein gene, wherein said portion comprises or flanks a mutation associated with predisposition to Parkinson's Disease. In particular, the oligonucleotides of the present invention will have a sequence that is complementary to a sequence from the alpha synuclein gene that includes or flanks base pair position 209. And in particular, this mutation is a change from guanine to adenine at this position.

Vectors comprising an isolated nucleic acid encoding a mutated synuclein gene will allow the production and isolation of the mutant protein in an appropriate host cell using techniques well known in the art. Alternatively, peptides may be chemically synthesized using techniques also well known in the art. Isolation of such a protein or peptides thereof will allow the study of the molecular mechanisms which lead to development of Parkinson's disease. Accordingly, the present invention also includes an isolated synuclein protein or peptide containing at least one mutation. In particular, this mutation is at a position corresponding to the fifty-third amino acid in the native alpha synuclein protein, and in particular, this mutation is an alanine to threonine substitution.

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Peptides corresponding to portions or the entirety of a synuclein gene may be useful as drugs for inhibiting the self-aggregation of mutant proteins that is thought to lead to Parkinson's disease. Accordingly, the present invention includes a method of testing peptides and other compounds for the ability to interfere with this self-aggregation. Self-aggregation can be tested using a number of established methods, including Congo red staining, electron microscopy pictures of amyloid fibrils, and circular dichroism (CD) spectrophotometry. Using a peptide derived from the alpha synuclein protein that includes the mutant THR amino acid at position 53 alone or in combination with a normal peptide may allow testing for drugs that can inhibit the aggregation or dissolve an aggregate. This procedure can be used to rapidly

identify agents that could be used in animal studies, clinical trials, or as diagnostic tools.

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Possession of isolated synuclein proteins or peptides will also allow the isolation of specific antibodies using techniques well known in the art. Such antibodies may distinguish a mutant synuclein protein from its wildtype counterpart, and therefor could also be used in diagnostic screens. Alternatively, such antibodies may also be used to inhibit the self-aggregation of proteins during the progression of Parkinson's disease. Accordingly, the present invention also includes antibodies specific for a mutated synuclein protein or peptide. It should be understood that useful derivatives of such antibodies, such as Fv fragments and Fab fragments, are also included.

The above aspects of the present invention will allow methods of detecting subjects at increased risk for Parkinson's Disease. Such a method comprises obtaining a sample comprising nucleic acids from the subjects, and detecting in the nucleic acids the presence of a mutation which is associated with Parkinson's disease. In particular, the mutation detected by the method of the present invention is located on human chromosome four, preferably in the alpha synuclein gene. In particular, the mutation causes an amino acid substitution at position 53 of the alpha synuclein gene, which is, in particular, an alanine to threonine substitution.

The detecting step of the method of the present invention may be accomplished several different ways as will

be described in further depth below. All such methods are well known to those of ordinary skill in the art.

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For instance, said detecting step may comprise combining a nucleotide probe which selectively hybridizes to a nucleic acid containing a mutation associated with a predisposition to Parkinson's disease, and detecting the presence of hybridization. Such a probe may be an oligonucleotide that is complementary to a portion of the synuclein gene, wherein said portion comprises the mutation. In particular, such an oligonucleotide is complementary to a mutated alpha synuclein gene having at least one mutation at base pair position 209. In particular, this mutation is a change from guanine to adenine.

The detecting step of the method of the present invention may also comprise amplifying a nucleic acid product comprising said mutation, and detecting the presence of said mutation in the amplified product using any nucleic acid sequencing procedure known in the art. Alternatively, the detecting step may comprise selectively amplifying a nucleic acid product comprising said mutation, and detecting the presence of amplification using any appropriate method known in the art. Such methods include gel electrophoresis of amplified nucleic acids, and detection of radiolabeled amplified nucleic acids using autoradiographic film or any other detection method known in the art.

The amplifying step of the present invention may be performed using the polymerase chain reaction (PCR), reverse transcriptase PCR (RTPCR), or any other type of PCR reaction

known in the art. Accordingly, such a step will comprise at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids. In particular, said amplifying step uses two oligonucleotides. And in particular, the two oligonucleotides have the sequences given in SEQ ID NOS 2 and 3.

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Alternatively, the detecting step of the method of the present invention comprises detecting the presence or absence of a restriction endonuclease site as detected by enzymatic digest of a nucleic acid sample. Such a detecting means will be possible when a mutation associated with a predisposition to Parkinson's disease results in a sequence having a new restriction endonuclease cleavage site, or loss of a native restriction endonuclease site. In particular, the mutation associated with Parkinson's disease results in the formation of a non-native Tsp45I restriction endonuclease site.

Alternatively, the detecting step of the present invention may be performed using a gene-specific primer and subsequent chain termination at the position of the mutation using DNA polymerase and labeled nucleotides or dideoxynucleotides. The presence of nucleic acids in which a dideoxynucleotide corresponding to the mutation of interest is incorporated at the appropriate position may be detected by any means known in the art, including detection of radiolabeled dideoxynucleotides using, for example, autoradiographic film, or detection of fluorescently-labeled dideoxynucleotides.

Since the methods and compounds of the present invention

will be useful in diagnostic screening procedures aimed at identifying individuals having a predisposition for Parkinson's disease, the present invention also includes diagnostic kits which include the compounds of the present invention in a form that allows such compounds to be used quickly and easily for the designated purpose.

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Finally, the inventors also contemplate that the isolated nucleic acid, oligonucleotides and antibodies of the present invention may eventually be used in methods directed at the correction or suppression of Parkinson's disease. For example, oligonucleotides or expression vectors designed from the synuclein nucleic acid sequences of the present invention may one day be used in antisense therapy directed at inhibiting expression of the mutated synuclein protein in patients with Parkinson's disease, or in individuals having a predisposition for Parkinson's disease. Similarly, antibodies specific for the mutated synuclein protein may be useful in therapies directed at inhibiting the selfaggregation of mutated proteins or peptides in patients having Parkinson's disease. Knowledge of gene(s) associated with the development of Parkinson's disease may also allow the design of transgenic animals which express the mutant gene(s). Such animals may serve as a useful disease model, allowing one to test the effects of candidate therapies and therapeutic compositions in the treatment or inhibition of Parkinson's disease.

A detailed description of the present invention is now provided, and should not be considered as limiting on the present invention as described above.

## 4. Brief Description of the Drawings

#### Figure 1.

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DNA sequence of the PCR product used for mutation detection (SEQ ID NO 1). Oligonuclectide primers are shown by arrows and the numerals 3 and 13 (SEQ ID NO 2 and 3). Intron sequence is shown in lower case and exon sequence in upper case. Amino acid translation of the exon is shown below the DNA sequence. The circled base represents the G209A change in the mutant allele. The resulting amino acid Ala53Thr change is represented by the circled amino acid. The newly created Tsp45 I site is indicated above the DNA sequence.

#### Figure 2.

Mutation analysis of the G209A change is shown in a subpedigree of the Italian kindred. Filled symbols represent affected individuals. Numerical identifiers, denote the individuals immediately above. *Tsp*45 I digestion of PCR products is shown at the bottom of the figure, and fragment sizes are indicated on the right in base pairs.

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Figure 3.

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Mutation analysis of the G209A change in RT PCR products (7). Lane 1: 100 bp ladder, lanes 2 and 3 normal control, lanes 4 and 5 PD patient, lane 6 negative control without RT enzyme. Sizes are indicated on the right in base pairs. Lanes 2 and 4 show uncut DNA and lanes 3 and 5 show DNA cut with Tsp45 I.

#### Figure 4.

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different species. Accession numbers for the sequences used were as follows: Homo sapiens Swiss-Prot P37840 (SEQ ID NO 4), Rattus norvegicus Swiss-Prot P37377 (SEQ ID NO 5), Bos taurus Swiss-Prot P33567 (SEQ ID NO 6), Serinus canaria genbank L33860 (SEQ ID NO 7), Torpedo californica Swiss-Prot P37379 (SEQ ID NO 8). Numbering on top of the alignments is according to the human sequence. Amino acid 53, which is the site of the Ala53Thr change, is circled.

#### Figure 5.

The pedigree of a large family with PD (3). The clinical and pathological features of some members of this kindred were previously reported.

#### Figure 6.

25 Multipoint LOD score analysis between chromosome 4q markers and the PD locus.

Figure 7.

A table of human synuclein clones identified from various databases. Columns labeled 5' and 3' show the sequence acquisition numbers. Clones were identified by homology to protein or nucleic acid sequence. Human gamma clones were identified by homology to known mouse and rat gamma synuclein sequences.

Figure 8.

Sequence of BAC clone 139A20 for human beta synuclein.

BAC clone was isolated using primers to known database sequences described in Figure 7. The sequence shown includes all coding exon sequences and some non-coding intronic sequences. (SEQ ID NO:11)

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Figure 9.

Sequence from the 5' end of BAC clone 174P13 for human gamma synuclein. The BAC clone was isolated with primers from the database sequences described in Figure 7.(SEQ ID NO:12)

Figure 10.

Sequence from the 3' end of BAC clone 174P13 for human gamma synuclein. BAC clone was isolated as described in Figure 9. The entire human gamma synuclein gene has now been sequenced and has been deposited in GenBank: accession number

AF044311.(SEQ ID NO: 13)

Figure 11.

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Sequence of exons 1-7 of the human alpha synuclein gene, plus some flanking intronic sequence for each exon. (SEQ ID NOs 14-19)

## 5. Detailed Description of the Invention

#### Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. For purposes of the present invention, the following terms are defined below.

This invention provides a method of diagnosing or predicting a predisposition to Parkinson's disease. The method comprises detecting in a sample from a subject the presence of a mutation, for example, in nucleotide position 209 of the human alpha synuclein gene. The presence of the mutation indicates the presence of or a predisposition to Parkinson's disease.

As used herein, the term "gene" primarily relates to a coding sequence, but can also include some or all of the surrounding or flanking regulatory regions or introns. The term "gene" specifically includes artificial or recombinant

genes created from cDNA or genomic DNA, including recombinant genes based upon splice variants.

As used herein, the term "synuclein" gene or protein may refer to the alpha synuclein gene or any homologue thereof.

A "homologue" is understood to mean any related gene or protein that is at least 25% homologous to the alpha synuclein gene or protein or performs a related function.

Preferably, a synuclein gene or protein refers to alpha, beta or gamma synuclein, but most preferably refers to alpha synuclein.

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As used herein, an "isolated nucleic acid" is a ribonucleic acid, deoxyribonucleic acid, or nucleic acid analog comprising a polynucleotide sequence that has been isolated or separated from sequences that are immediately contiguous, i.e. on the 5' and 3' ends, in the naturally occurring genome of the organism from which it is derived. The term therefor includes, for example, a recombinant nucleic acid which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule independent from any other sequences.

An isolated nucleic acid of the present invention may be "operatively linked" to an expression control sequence or regulatory region. As used herein, "operatively linked" means that the components are joined in such a way that the expression, transcription or translation of the sequence is under the influence or control of the regulatory region.

As used herein, a "predisposition" to Parkinson's

disease means an increased probability of developing

Parkinson's disease during the subject's lifetime as compared
to the average individual.

Pertaining to this probability, a LOD score is a measure of genetic linkage used herein, defined as the logic ratio of the probability that the data would have arisen if the loci are linked to the probability that the data could have arisen from unlinked loci. The conventional threshold for declaring linkage is a LOD score of 3.0, that is, a 1000:1 ratio (which must be compared with the 50:1 probability that any random pair of loci will be unlinked).

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As used herein, reference to "base pair position" or "amino acid position" when referring to an isolated nucleic acid, probe, protein or peptide always indicates the relative position in the native gene or protein.

A "probe" refers to a nucleic acid which has sufficient nucleotides surrounding the codons at the mutation positions to distinguish the nucleic acid from nucleic acids encoding non-related genes. The specific length of the nucleic acid is a matter of routine choice based on the desired function of the sequence. For example, if one is making probes to detect the mutation in base pair position 209, the length of the nucleic acid is preferably small, but must be long enough to prevent hybridization to undesired background sequences. However, if the desired hybridization is to a nucleic acid which has been amplified, background hybridization is less of a concern and a smaller probe can be used. In general, such a probe will be between 10 and 100 nucleotides, especially

between 10 and 40 and preferably between 15 and 25 nucleotides in length. It is apparent to one of skill in the art that nucleotide substitutions, deletions, and additions may be incorporated into the polynucleotides of the invention. However, such nucleotide substitutions, deletions, and additions should not substantially disrupt the ability of the polynucleotide to hybridize under conditions that are sufficiently stringent to result in specific hybridization.

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As used herein with respect to genes, "the term "normal" refers to a gene which encodes a normal protein. As used herein with respect to proteins, the term "normal" means a protein which performs its usual or normal physiological role and which is not associated with, or causative of, a pathogenic condition or state. Therefor, the term "normal" is generally synonomous with the phrase "wild type".

For any given gene or corresponding protein, a multiplicity of normal allelic variants may exist, none of which is associated with the development of a pathogenic condition or disease state. Such normal allelic variants include, but are not limited to, variants in which one or more nucleotide substitutions do not result in a change in the encoded amino acid sequence.

As used herein, the term "mutation" generally refers to a mutation in a gene that is associated with a predisposition to Parkinson's disease. "Mutant" can specifically refer to a mutation at nucleotide position 209 of the synuclein gene, and is in particularly a G to A transition. However, other mutations in the synuclein gene or other genes which are

associated with a predisposition to Parkinson's disease are also encompassed. Furthermore, the term "mutation" is not limited to transition mutations, but can also mean a deletion, insertion or transversion as well.

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The term "mutant", as it applies to synuclein genes, is not intended to embrace sequence variants which, due to the degeneracy of the genetic code, encode proteins identical to the normal sequences disclosed or otherwise enabled herein; nor is it intended to embrace sequence variants which, although they encode different proteins, encode proteins which are functionally equivalent to normal synuclein proteins. The term "mutant" means a protein which does not perform its usual or normal physiological role and which is associated with, or causative of, a pathogenic condition or state.

Since a mutation can be a substitution, deletion or insertion, a mutated synuclein "protein" is understood to refer to the amino acid sequence resulting from any such mutation whether the resulting protein is shorter, longer or modified, i.e. due to an alteration in reading frame or generation of stop codon. In addition, "peptide" is understood to refer to a portion of the mutated protein that is preferably at least five base pairs long, and more preferably at least 10 base pairs long. This portion may be derived from the amino or carboxyl terminus, or it may be an internal portion of the full length protein. As such, a peptide may be chemically synthesized using any method known in the art, or may be made using a recombinant DNA technology

and an appropriate purification scheme or isolated from the native protein using enzymatic digestion.

As used herein, the term "substantially pure" means a preparation which is at least 60% by weight the compound of interest. Preferably the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate method, i.e. column chromotography, gel electrophoresis or HPLC analysis.

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"Specific or selective hybridization" as used herein means the formation of hybrids between a probe nucleic acid (e.g., a nucleic acid which may include substitutions, deletions, and/or additions) and a specific target nucleic acid (e.g., a nucleic acid having the mutated sequence), wherein the probe preferentially hybridizes to the specific target such that, for example, a band corresponding to the mutated DNA or restriction fragment thereof can be identified on a Southern blot, whereas a corresponding normal or wild-type DNA is not identified or can be discriminated from a variant DNA on the basis of signal intensity. Hybridization probes capable of specific hybridization to detect a single-base mismatch may be designed according to methods known in the art (13-17).

"Stringent" as it refers to hybridization conditions is a term of art understood by those of ordinary skill to refer to those conditions of temperature, chaotrophic acids, buffer and ionic strength which permit hybridization of a particular nucleic acid sequence to its complementary sequence and not

to substantially different sequences. The exact conditions which constitute "stringent" conditions depend on the nature of the nucleic acid sequence, the length of the sequence, and the frequency of occurrence of subsets of that sequence within other non-identical sequences. By varying hybridization conditions from a level of stringency at which non-specific hybridization occurs to a level at which only specific hybridization occurs, one of ordinary skill in the art can, without undue experimentation, determine conditions which will allow a given sequence to hybridize only with complementary sequences.

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Suitable ranges of stringency conditions are described in Sambrook et al. (13). Hybridization conditions, depending on the length and commonality of a sequence, may include temperatures of 20°C-65°C and ionic strengths from 5X to 0.1X SSC. Highly stringent hybridization conditions may include temperatures as low as 40°C-42°C (when denaturants such as formamide are included) or up to 60°C-65°C in ionic strengths as low as 0.1X SSC. These ranges are, however, only illustrative and, depending on the nature of the target sequence, and possible future technological developments, may be more stringent than necessary. Appropriate conditions may be determined for each specific nucleic acid sequence or oligonucleotide probe using standard control and a level of experimentation that is not considered to be undue by those of skill in the art.

As discussed below in greater detail, the mutation can be detected by many methods. For example, the detecting step can comprise combining a nucleotide probe capable of selectively hybridizing to a nucleic acid containing the mutation with a nucleic acid in the sample and detecting the presence of hybridization. Additionally, the detecting step can comprise amplifying the nucleotides surrounding and including the mutation and detecting the presence of the mutation in the amplified product, or selectively amplifying the nucleotides of the mutation and detecting the presence of amplification. Finally, the detecting step can comprise detecting the presence or absence of a restriction fragment created by an enzyme digest of the sample nucleic acid, or any other detection means known in the art.

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#### Detection Techniques

Once the location of a PD-relevant mutation is known, the methods to detect such a mutation are standard in the art. The sequence of various nucleotide probes can be determined from the known sequence of the relevant gene, especially the sequences surrounding the mutation.

Detection of point mutations using direct probing involves the use of oligonucleotide probes which may be prepared, for example, synthetically or by nick translation. The probes may be suitably labeled using, for example, a radio label, enzyme label, fluorescent label, biotin-avidin label and the like for subsequent visualization by any appropriate assay, i.e. Southern blot hybridization. In this procedure, the labeled

probe is reacted with sample DNA that is bound, for example, to a nylon filter under conditions such that only fully complementary sequences hybridize. The areas that carry DNA sequences complementary to the labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling may then be visualized, for example, by autoradiography.

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Methods of manipulating hybridization conditions to achieve varying degrees of specificity are well known in the art. For example, tetra-alkyl ammonium salts may be used to bind selectively to A-T base pairs, thus displacing the dissociation equilibrium and raising the melting temperature. At 3M Me 4NCl, this is sufficient to shift the melting temperature to that of G-C pairs. This results in a marked sharpening of the melting profile. The stringency of hybridization in such an experiment is usually 5°C below the Ti (the irreversible melting temperature of the hybrid formed between the probe and its target sequence) for the given chain length. For a 20mer oligonucleotide probe, the recommended hybridization temperature is about 58°C. The washing temperatures are unique to the sequence under investigation and need to be optimized for each variant.

There are certainly other ways known in the art for adjusting hybridization conditions in view of desired specificity. For instance, although hybridization may be carried out in accordance with conventional hybridization methods under suitable conditions with respect to e.g. stringency, incubation time, temperature, etc, the choice of

conditions will depend on the desired degree of complementarity between the fragments to be hybridized. A high degree of complementarity requires more stringent conditions such as low salt concentrations, low ionic strength of the buffer and higher temperatures, whereas a low degree of complementarity requires less stringent conditions, e.g. higher salt concentration, higher ionic strength of the buffer or lower temperatures, for the hybridization to take place.

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The support to which DNA or RNA fragments of the sample to be analyzed are bound in denatured form is preferably a solid support and may have any convenient shape. Thus, it may, for instance, be in the form of a plate, e.g. a thin layer or a microtiter plate, a strip, a solid particle e.g. in the form of a bead such as a latex bead, a filter, a film or paper. The solid support may be composed of a polymer, preferably nylon or nitrocellulose.

Alternative probing techniques, such as ligase chain reaction (LCR), may involve the use of mismatch probes, i.e., probes which are fully complementary with the target except at the point of the mutation. The target sequence is then allowed to hybridize both with oligonucleotides which are fully complementary and have oligonucleotides containing a mismatch, under conditions which will distinguish between the two. By manipulating the reaction conditions according to the above considerations, it is possible to obtain hybridization only where there is full complementarity. If a mismatch is present there is significantly reduced hybridization.

The polymerase chain reaction (PCR) is a technique that amplifies specific DNA sequences with remarkable efficiency. Repeated cycles of denaturation, primer annealing and extension carried out with Taq polymerase, e.g., a heat stable DNA polymerase, leads to exponential increases in the concentration of desired DNA sequences. Given a knowledge of the nucleotide sequence of the mutations, synthetic oligonucleotides can be prepared which are complementary to sequences which flank the DNA of interest. Each oligonucleotide is complementary to one of the two strands. The DNA is denatured at high temperatures (e.g., 95°C) and then reannealed in the presence of a large molar excess of oligonucleotides. The oligonucleotides, oriented with their 3' ends pointing towards each other, hybridize to opposite strands of the target sequence and prime enzymatic extension along the nucleic acid template in the presence of the four deoxyribonucleotide triphosphates. The end product is then denatured again for another cycle. After this three-step cycle has been repeated several times, amplification of a DNA segment by more than one million-fold can be achieved. The resulting DNA may then be directly sequenced in order to locate any genetic alteration. Alternatively, it may be possible to prepare oligonucleotides that will only bind to altered DNA, so that PCR will only result in multiplication of the DNA if the mutation is present. Following PCR, direct visualization or allele-specific oligonucleotide hybridization (18) may be used to detect the Parkinson's disease point mutation. Alternatively, PCR may be followed

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by restriction endonuclease digestion with subsequent analysis of the resultant products.

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As shown in the examples, the substitution of G for A at base pair 209 of the synuclein, results in the gain of a Tsp45I site. The gain of this restriction endonuclease recognition site facilitates the detection of the Parkinson's disease mutation using restriction fragment length polymorphism (RFLP) analysis or by detection of the presence or absence of the restriction site in a PCR product that spans base pair position 209.

For RFLP analysis, DNA is obtained, for example from the blood cells of the subject suspected of having Parkinson's disease and from a normal subject, is digested with a restriction endonuclease, and subsequently separated on the basis of size by agarose gel electrophoresis. The Southern technique can then be used to detect, by hybridization with labeled probes, the products of endonuclease digestion. The patterns obtained from the Southern blot can then be compared. Using such an approach, an additional restriction endonuclease site, such as a Tsp45I site, is detected by determining the number of bands detected and comparing this number to the normal subject.

The creation of a new restriction site as a result of a nucleotide substitution at a disclosed mutation site can be readily determined by reference to the genetic code and a list of nucleotide sequences recognized by restriction endonucleases (19).

In general, primers for PCR are usually about 20 bp in

length, and are most preferably 15-25 bp. Denaturation of strands usually takes place at 94°C. and extension from the primers is usually at 72°C. The annealing temperature varies according to the sequence under investigation. Examples of reaction times are: 20 mins denaturing; 35 cycles of 2 min, 1 min, 1 min for annealing, extension and denaturation; and finally a 5 min extension step.

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PCR "amplification of specific alleles" (PASA) may also be used to detect the presence of the PD mutation. PASA is a rapid method of detecting single-base mutations or polymorphisms (22-28). PASA (also known as allele specific amplification) involves amplification with two oligonucleotide primers such that one is allele-specific. The desired allele is efficiently amplified, while the other allele(s) is poorly amplified because it mismatches with a base at or near the 3' end of the allele-specific primer. Thus, PASA or the related method of PAMSA may be used to specifically amplify the mutation sequences of the invention. Where such amplification is done on genetic material (or RNA) obtained from an individual, it can serve as a method of detecting the presence of the mutations.

As mentioned above, a method known as ligase chain reaction (LCR) can be used to successfully detect a single-base substitution (29, 30). LCR probes may be combined or multiplexed for simultaneously screening for multiple different mutations. Thus, LCR can be particularly useful where multiple mutations are predictive of the same disease.

Finally, the Parkinson's disease mutation of the present invention may also be detected using chain termination with labeled dideoxynucleotides. For instance, U.S. Patent No. 5,047,519 to Hobbs et al. discloses fluorescently-labeled nucleotides as chain-terminating substrates for a fluorescence-based DNA sequencing method. With such substrates and knowledge of the gene sequence of interest, it is possible to design an assay using a gene-specific primer to initiate a polymerase reaction immediately flanking the position of the mutation, employing color-coded dideoxynucleotide terminators such that the specific nucleotide at the position of the mutation may be easily determined via a colorimetric assay.

### 15 Transgenic Animals and Cell Lines

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Having identified subjects having a predisposition to Parkinson's disease associated with a specific mutation, the subjects can participate in the screening of putative agents capable of treating Parkinson's disease. This method comprises administering the test agent to the subject, which may be a human, which has a mutation in a gene associated with Parkinson's disease and monitoring the effect of the agent on the subject's condition. If the symptoms of Parkinson's disease improve, the agent can be used as a treatment for the disease.

In addition, it is possible to develop transgenic model systems and/or cell lines containing the mutated nucleic acid(s) for use, for example, as model systems for screening

for drugs and evaluating drug efficiency. Additionally, such model systems provide a tool for defining the underlying biochemistry of, for instance, the mutated synuclein gene, thereby providing a rationale for drug design.

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One approach to creating transgenic animals is to mutate the animal gene of interest by in vivo mutagenesis, transfer the mutant gene into embryonic stem cells by DNA transfection and inject the embryonic stem cells into blastocysts in order to retrieve offspring which carry the disease-causing mutation (31). Alternatively, the technique of microinjection of the mutated gene, into a one-cell embryo followed by incubation in a foster mother can be used. Alternatively, viral vectors, e.g., Adeno-associated virus, can be used to deliver the mutated gene to a stem cell, or may be used to target specific cells of a fully developed animal (32,33).

#### Antibodies and Recombinant Expression of Polypeptides

When the mutated gene product is a polypeptide, e.g. the 209 mutation, it can be used to prepare antisera and monoclonal antibodies using, for example, the method of Kohler and Milstein (34). Such monoclonal antibodies could then form the basis of a diagnostic test, or may even be useful in therapies directed toward inhibiting the action of the mutant protein in a patient with Parkinson's disease.

Mutant polypeptides can also be used to immunize an animal for the production of polyclonal antiserum (35). For example, a recombinantly produced fragment of a variant polypeptide can be injected into a mouse along with an

adjuvant so as to generate an immune response. Murine immunoglobulins which specifically bind the recombinant fragment can be harvested from the immunized mouse as an antiserum, and may be further purified by affinity chromatography or other means. Additionally, spleen cells are harvested from the mouse and fused to myeloma cells to produce a bank of antibody-secreting hybridoma cells, which can then be screened for clones that secrete immunoglobulins which bind the recombinantly produced fragment with an increased affinity. More specifically, immunoglobulins that selectively bind to the variant polypeptides but poorly or not at all to wild-type polypeptides are selected, either by pre-absorption with wild-type proteins or by screening of hybridoma cell lines for specific idiotypes that bind the variant but not wild-type.

These antibodies can be used to screen protein and tissue samples for the presence of mutated proteins. A colored enzymatic reaction occurs when the specific antibody remains bound to its target protein, in situ, after thorough washing, as directed by established protocols.

#### Gene expression

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The nucleic acid sequences of the present invention will be capable of expressing the desired mutant or normal polypeptides in an appropriate host cell. For expression in host cells, the DNA sequences of the present invention will be operably linked to, i.e., positioned to ensure the functioning of, an expression control sequence. For example,

such polynucleotides can include a promoter, a transcription termination site (polyadenylation site in eukaryotic expression hosts), a ribosome binding site, and, optionally, an enhancer for use in eukaryotic expression hosts. In addition, the DNA sequence of the present invention may also be fused such that the reading frame is conserved to an appropriate signal sequence to facilitate export of the encoded protein across the cell membrane.

Expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. A variety of suitable expression vectors are disclosed in Sambrook et al. (13). Commonly, expression vectors will contain selection markers, e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells transformed with the desired DNA sequences.

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E. coli is one prokaryotic host that is particularly useful for cloning and expression of the DNA sequences of the present invention because of the wide variety of available expression systems. Vectors suitable for use in E. coli are known and are commercially available, i.e. pBR322 (13), pBLUESCRIPT (Stratagene), etc. Also, a variety of different types of expression systems may be used, including plasmids, cosmids, bacteriophage lambda, etc. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilus, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. Expression vectors for use in prokaryotic host cells will typically contain expression

control sequences compatible with the host cell (e.g., an origin of replication). In addition, any of a variety of well-known promoters may be used, such as the lactose promoter system, a tryptophan (Trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. A promoter may optionally contain an operator sequence for regulatable gene expression, and will have a ribosome binding site sequence for the initiation of translation.

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In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (36). Vectors for use in eukaryotic cells are known and commercially available, i.e. pcDNA3 (Invitrogen). Eukaryotic cells are actually preferred, and a number of suitable host cell lines capable of secreting intact human proteins have been developed in the art, including CHO cells, COS cells, HeLa cells, myeloma cell lines, Jurkat cells, etc. Promoters for use in eukaryotic vectors may be cell-specific, or capable of being expressed in a wide variety of cells, i.e. viral promoters.

Expression vectors of the present invention (e.g., comprising nucleic acid sequences encoding a mutant or normal polypeptide) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts.

#### <u>Kits</u>

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The method lends itself readily to the formulation of test kits which can be utilized in diagnosis. Such a kit would comprise a carrier compartmentalized to receive in close confinement one or more containers wherein a first container may contain suitably labeled DNA probes. Other containers may contain reagents useful in the localization of the labeled probes, such as enzyme substrates. Still other containers may contain restriction enzymes (such as Tsp45I), buffers, etc., together with instructions for use.

#### DESCRIPTION OF THE INVENTION

#### Detailed Description of the Preferred Embodiments

The following laboratory procedures were used:

DNA samples were collected upon informed consent. High molecular weight genomic DNA was isolated from whole-blood lysate by methods previously described (38). Genotyping was performed as previously described (39). Pairwise linkage analysis was performed using the MLINK program of the FASTLINK package (40-42). Allele frequencies were used as reported in the Genomic Data Base (http://gdbwww.gdb.org) and the Cooperative Human Linkage Consortium (CHLC) database (http://www.chlc.org). Multipoint analysis was performed using the LINKMAP program of the FASTLINK package. For the multipoint analysis allele frequencies were set to 1/n where n equals the number of alleles observed. In the two point analysis LOD scores were calculated for both the reported and the 1/n allele frequencies with minimal effect on the

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maximum LOD score observed. Simulations of multipoint analysis in a subset of the pedigree with different allele frequencies similarly indicated no significant effect on the scores calculated. Maximum LOD scores as shown were observed for the heterozygote and homozygote disease allele penetrance set to 0.99, which is similar to the PD allele penetrance previously reported ranging from 0.88 to 0.94 (3). All unaffected individuals used in the study were of age above the mean for onset of illness. Disease allele frequency was set to 0.0001. Distances and order of genetic markers were set as reported in the CHLC database. Overlapping three point analysis was performed for markers D4S2361, D4S1647, D4S421 and the PD locus. The 12 allele D4S2380 locus was not included because of prohibitive time run. Multipoint analysis was performed on an IBM SP2 parallel computer and the SGI Challenge machine.

For mutation analysis genomic DNA was amplified with oligonucleotides (3): 5' GCTAATCAGCAATTTAAGGCTAG 3' (SEQ ID NO 2) and (13): 5' GATATGTTCTTAGATGCTCAG 3' (SEQ ID NO 3) of genbank ID: U46898, under standard PCR conditions. Sequence analysis was performed using the Perkin Elmer dye terminator cycle sequencing kit on an ABI 373 fluorescent sequencer (ABI, Foster City, CA). Restriction digestion was performed following the PCR with Tsp45 I according to manufacturer's protocol (New England Biolabs, Beverly, MA). The digested PCR products were electrophoresed on a 6% Visigel (Stratagene, La Jolla, CA), and visualized by ethidium

bromide staining. Pedigree structure in Figure 2 has been slightly modified in order to protect patient confidentiality.

Total RNA was extracted from the lymphoblastoid cell line of an affected individual and first strand synthesis was performed by oligo dT priming (Gibco BRL, Gaithersburg, MD). Primers (1F) 5' ACGACAGTGTGGTGTAAAGG 3' (SEQ ID NO 9) and (13R) 5' AACATCTGTCAGCAGATCTC 3' (SEQ ID NO 10) corresponding to nucleotides 21-40 and 520-501 of genbank L08850 were used to amplify a product of 500 bp containing the mutation at nucleotide 209. PCR products were subjected to restriction digestion by Tsp45 I. The mutation at nt 209 creates a novel Tsp45 I site (Figure 1), so that the normal allele will be restricted in 4 fragments of 249, 218, 24 and 9 bp, where the mutant allele will have 5 fragments of 249, 185, 33, 24 and 9 bp of size, as shown in Figure 3. Size standards used, were the 100 bp ladder (Gibco BRL, Gaithersburg, MD).

## Example 1

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In an effort to identify a genetic locus responsible for Parkinson's disease, we performed a genome scan in a large kindred of Italian descent with pathologically confirmed PD (Figure 5). The kindred originated in the town of Contursi in the Salerno province of Southern Italy (3). Some members emigrated to the United States, Germany and other countries. The extended family pedigree consists of 592 members with 60 individuals affected by PD. The average age of onset for the

illness in this pedigree (Figure 5) has been shown to be 46  $\pm$  13 years. One hundred and fourty genetic markers were typed in this pedigree at an average spacing of about 20 cm. Genetic markers at the cytogenetic location 4q21-q23 were the only ones to show linkage to the disease phenotype with a 2max=6.00 at theta=0.00 for marker D4S2380I (see Table 1).

Table 1. Two point LOD scores between chromosome 4q markers and the PD locus

		Two-poin	t LOD	scores	at recor	mbinatio	n fract	ions of	:	
	Locus	0.00	0.01	0.05	0.10	0.20	0.30	0.40	$Z_{max}$	$\theta_{\text{max}}$
15	D4S2361	-5.60	-0.83	0.30	0.54	0.43	0.21	0.06	0.55	0.12
	D4S2380	6.00	5.90	5.30	4.60	3.00	1.50	0.50	6.00	0.00
20	D4S1647	5.22	5.07	4.47	3.71	2.26	1.05	0.30	5.22	0.00
	D4S421	-2.42	0.45	0.77	0.65	0.38	0.22	0.09	0.77	0.05

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Recombinations between the disease phenotype and genetic markers were observed in the proximal region for marker D4S2361 and in the distal region for marker D4S2361 and in the distal region for marker D4S2380 and D4S1647 showed no obligate recombination events in the affected individuals.

Multipoint LOD score analysis between markers D4S2361-13cM-D4S1647-3cM-D4S421 and the disease locus places the PD gene between markers D4S2361 and D4S421 at a recombination distance of 0.00 cM from marker D4S1647 with a Zmax=6.04 (Figure 6). This location is favored from the alternative genetic intervals by a difference in the LOD score of greater

than three LOD units.

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Although expansions of unstable trinuclectide repeats are found in a number of human neurogenerative conditions, there is no evidence for an association of a CAG trinucleotide repeat expansion in families with PD (43). In addition, genetic linkage studies in other families with PD-like illnesses do not support the involvement of several candidate genes (glutathione peroxidase, tyrosine hydroxylase, brain-derived neurotrophic factor, catalase, amyloid precursor protein, CuZn superoxide dismutase and debrisoquinone 4-hydroxylase) in the etiology of the disorder (44). Genes previously mapped in the general region of linkage include the loci for alcohol dehydrogenase, formaldehyde dehydrogenase, synuclein, UDP-N-acetylglycosamine phosphotransferase and others.

Our localization of a PD susceptibility gene represents the first genetic locus linked to PD. Other distinct clinicopathological entities associated with parksonian features are probably linked to other genetic loci. For example, Wilhelmsen-Lynch disease (disinhibition-dementia-parkinsonian-amyotrophy complex) is linked to the 17q21-q22 chromosomal region (45). If the pathogenesis of diseases affecting the nigrostriatal pathway includes environmental influences, then a range of mutations affecting vulnerable sites in the electron transport chain or enzyme polymorphisms influencing neurotoxin metabolism may vary the penetrance of PD by altering an individual's resistance to exogenous or endogenous agents. However, our finding of a highly

penetrant genetic locus linked to PD suggested that abnormalities of a single gene may be sufficient to cause Parkinson's disease.

# 5 Example 2

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In an effort to identify a specific gene between markers D4S2361 and D4S421 that is associated with predisposition to Parkinson's disease, we conducted sequence analysis of candidate genes in this region.

Alpha synuclein, a presynaptic nerve terminal protein, was originally identified as the precursor protein for the NAC peptide, a non beta amyloid component of Alzheimer's disease (AD) amyloid plaques (4). The human alpha synuclein gene was previously mapped in the 4q21-q22 region (5). We refined the mapping, and determined that the alpha synuclein gene is located within the non-excluded region harboring the PD gene in the Italian kindred. Thus alpha synuclein represented an excellent candidate locus for PD.

Sequence analysis of the fourth exon of the alpha synuclein gene revealed a single base pair G209A change from the published sequence of the gene (GenBank ID L08850), which results in an Ala53Thr substitution and the creation of a novel Tsp45 I restriction site (Figure 1). Mutation analysis for the G209A change in the Italian kindred shows complete segregation with the PD phenotype with exception of

individual 30 (Figure 2), who is affected but not carrying this mutation. This individual apparently inherited a different PD mutation from his father, as we have shown that he shares a genetic haplotype with his unaffected maternal uncle, individual 3, for genetic markers in the PD linkage region.

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The frequency of this variation was studied in two general population samples, one consisting of 120 chromosomes of the parents of the CEPH reference families, and the other consisting of 194 chromosomes of unrelated individuals from the blood bank in Salerno, Italy, a city near the town from which the family originated. Of these 314 general population chromosomes none was found to carry the G209A mutation. Fifty two patients of Italian descent with sporadic PD were also screened for the mutation (Figure 2), along with 5 probands from previously unpublished Greek families with PD. The Ala53Thr change was found to be present in three of the Greek kindreds and it segregated with the PD phenotype. those three Greek kindreds it is worth noting that the age of onset for the disease is relatively early, ranging from the mid 30's to the mid 50's. Extended haplotype analysis of the Greek kindreds and the Italian PD family suggests that the mutations arose independently on different ancestral chromosomes. The finding of the Ala53Thr substitution in four independent PD families and its absence from 314 control chromosomes provides the strongest genetic evidence that this

mutation in the human alpha synuclein gene is causative for the PD phenotype in these families.

We have also demonstrated by RT PCR that the mutant allele is transcribed in the lymphoblast cell line of an affected individual from the Italian kindred (Figure 3) (7). Thus, it is reasonable to assume that the mutant protein is indeed expressed.

## Example 3.

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Since homologous genes that are related to the alpha synuclein protein have been identified in other species, it seemed reasonable to assume that homologues of alpha synuclein would exist in humans as well. In fact, human beta synuclein has previously been described (46), and is approximately 60% similar to alpha synuclein at the protein level.

We set out to identify other related homologues by searching various databases for homologous genes and proteins. Protein sequence databases searched included the NR (non-redundant) and "month" databases of Genbank and Swiss Prot. Nucleotide databases included NR, month, dbstf, GSS (Genome Sequence Service) and EPD (eurkaryotic Promoter Database). Several human clones were identified and characterized as alpha, beta and gamma clones as shown in Figure 7. Potential gamma clones were identified on the basis of homology to known rat and mouse sequences. Although

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gamma synuclein has been identified in species other than human, this is the first identification of the corresponding gamma synuclein from humans.

Using two primers sets designed from known database sequences (5'ATGTCTTCAAGAAGGGCTTC3'; 5'CCTTGGTCTTCTCAGCTGCT3' and 5'AGCGTGGATGACCTGAAGAG3'; 5'AGCACAGGTGGACAGGCCAAG3'), we have isolated two BAC clones, 139A20 and 174P13, from a Genome System commercial Bacterial Artificial Chromosome library (St. Louis, MO) which contain the human beta and gamma synuclein genes, respectively. The beta gene contained one clone 139A20 has been sequenced as shown in Figure 8 (SEQ ID NO 11), which contains all coding exon sequences and some additional non-coding intronic sequence. The gamma clone 174P13 has been sequenced and is available in GenBank: accession number AF044311. Sequence from the 5' end is given in Figure 9 (SEQ ID NO 12), and sequence from the 3' end is given in Figure 10 (SEQ ID NO 13). The human alpha synuclein gene has also been sequenced as shown in Figure 11, which provides the sequence of each separate exon region with some additional flanking intronic sequence for each exon. (SEO ID NOs 14-19)

The three human homologues are highly conserved at the protein level. The alpha and beta human homologues have about 60.4% similarity. And the gamma homologue is about 38.3% and 32.8% similar to the alpha and beta homologues, respectively, based on the portion of the coding sequence

that we have obtained thus far. Thus, it is reasonable to presume that mutations in either the beta or gamma synuclein gene may also result in Parkinson's disease.

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- 47. Polymeropoulos et al. (1997) Science 276:2045-2047, which is relied upon and hereby expressly incorporated by reference herein.
- 48. Lavedan et al. (1998) in press, which is relied upon and hereby expressly incorporated by reference herein.
- 49. This application is based on provisional application number 60/505,684 filed June 25, 1997 which is relied upon and hereby expressly incorporated by reference herein.

# SEQUENCE LISTING

	(1) GENERAL INFORMATION:
5	(i) APPLICANT: Polymeropoulus, Mihael Lavedan, Christian Leroy, Elisabeth
10	Nussbaum, Robert Johnson, William Duvoisin, Roger
15	(ii) TITLE OF INVENTION: Cloning of a gene mutation for Parkinson's disease
10	(iii) NUMBER OF SEQUENCES: 10
20	<ul><li>(iv) CORRESPONDENCE ADDRESS:</li><li>(A) ADDRESSEE: SPENCER &amp; FRANK</li><li>(B) STREET: 1100 New York Ave. Suite 300 East</li><li>(C) CITY: Washington</li></ul>
25	(D) STATE: D.C. (E) COUNTRY: USA (F) ZIP: 20005-3955
	<ul><li>(V) COMPUTER READABLE FORM:</li><li>(A) MEDIUM TYPE: Floppy disk</li><li>(B) COMPUTER: IBM PC compatible</li><li>(C) OPERATING SYSTEM: PC-DOS/MS-DOS</li></ul>
30	(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
35	<ul><li>(vi) CURRENT APPLICATION DATA:</li><li>(A) APPLICATION NUMBER:</li><li>(B) FILING DATE: 25-JUN-1998</li><li>(C) CLASSIFICATION:</li></ul>
4 O	<pre>(viii) ATTORNEY/AGENT INFORMATION:</pre>
45	<pre>(ix) TELECOMMUNICATION INFORMATION:     (A) TELEPHONE: (202)414-4000     (B) TELEFAX: (202)414-4040</pre>
10	(2) INFORMATION FOR SEQ ID NO:1:
50	<ul> <li>(i) SEQUENCE CHARACTERISTICS.</li> <li>(A) LENGTH: 216 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: double</li> <li>(D) TOPOLOGY: linear</li> </ul>

	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
5	(iv) ANTI-SENSE: NO	
10	<pre>(vii) IMMEDIATE SOURCE:     (B) CLONE: alpha synuclein gene/ exon 4 region</pre>	
- "	(viii) POSITION IN GENOME:  (A) CHROMOSOME/SEGMENT: 4  (B) MAP POSITION: 4q21-q22	
15		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:	
20	GCTAATCAGC AATTTAAGGC TAGCTTGAGA CTTATGTCTT GAATTTGTTT TTGTAGGCTC	60
	CAAAACCAAG GAGGGAGTGG TGCATGGTGT GACAACAGGT AAGCTCCATT GTGCTTATAT	120
	CAAAGATGAT ATNTAAAGTAT CTAGTGATTA GTGTGGCCCA GTATCAAGAT TCCTATGAA	181
25	ATTGTAAAACA ATCACTGAGC ATCTAAGAAC ATATC	216
	(2) INFORMATION FOR SEQ ID NO:2:	
30	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 22 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
35	<pre>(ii) MOLECULE TYPE: other nucleic acid      (A) DESCRIPTION: /desc = "primer #3"</pre>	
	(iii) HYPOTHETICAL: NO	
<b>:</b> (1		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:	
45	GCTAATCAGC AATTTAGGCT AG	22
	(2) INFORMATION FOR SEQ ID NO:3:	
50	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 21 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	

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BNSDOCID +WO 4859050A1 Lix

	(ii)	MOLE( (A)	CULE DESC								3 "						
5	(iii)	НҮРОТ	THET 1	CAL:	: <b>N</b> O												
10	(xi)	SEQUI	ENCE	DESC	CRIPT	NOIT	: SE(	D ID	NO:3	3:							
	CTATACAAC	TA AE	CTAC	SAGT	С												21
	(2) INFO	TAMS	ON FO	OR S	EQ II	ON C	:4:										
15	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 140 amino acids</li> <li>(B) TYPE: amino acid</li> <li>(C) STRANDEDNESS: not relevant</li> <li>(D) TOPOLOGY: not relevant</li> </ul>																
20	(ii)	MOLE	CULE	TYP	E: p	epti	de										
	(iii)	нүро	THET	ICAL	: NO												
25	(iv)	ANTI	-SEN	SE:	NO												
30	<pre>(vi) ORIGINAL SOURCE:     (A) ORGANISM: Homo sapiens     (C) INDIVIDUAL ISOLATE: Swiss-Prot P37840</pre>																
30	(vii)		DIAT CLC				nucl	ein	prot	ein							
35	(xi)	SEQU	JENCE	E DES	CRIE	10IT	1: SE	Q II	NO:	4:							
4.0	Met 1	Asp	Val	Phe	Met 5	Lys	Gly	Leu	Ser	Lys 10	Ala	Lys	Glu	Gly	Val 15	Val	
40	Ala	a Ala	Ala	Glu 20	Lys	Thr	Lys	Gln	Gly 25	Val	Ala	Glu	Ala	Ala 30	Gly	Lys	
45	Thi	. Lys	Glu 35	Gly	Val	Leu	Tyr	Val 40	Gly	Ser	Lys	Thr	Lys 45	Glu	Gly	Val	
	Va.	l His 50	Gly	Val	Ala	Thr	Val 55	Ala	Glu	Lys	Thr	Lys 60	Glu	Gln	Val	Thr	
50	As:	n Val	Gly	Gly	Ala	Val 70	Val	Thr	Gly	Val	Thr 75	Ala	Val	Ala	Gln	Lys 80	
	Th	r Val	Glu	Gly	Ala 85	Gly	Ser	Ile	Ala	Ala 90	Ala	Thr	Gly	Phe	Val 95	Lys	

	Lys	Asp	Gln	Leu 100	Gly	Lys	Asn	Clu	Glu 105	Gly	Ala	Pro	Gln	Glu 110	Gly	Ile
E,	Leu	Glu	Asp 115	Met	Pro	Val	Asp	Pro 120	Asp	Asn	Glu	Ala	Tyr 125	Glu	Met	Pro
	Ser	Glu 130	Glu	Gly	Tyr	Gln	Asp 135	Tyr	Glu	Pro	Glu	Ala 140				
10	(2) INFO	RMATI	ON F	FOR S	SEQ 1	D NC	):5:									
15	(i)	(B)	JENCE LEN TYI STI TOI	IGTH: PE: & RANDE	140 amino EDNES	ami aci SS: r	no a ld not r	acids relev								
	(ii)	MOLE	ECULE	E TYF	PE: p	pepti	de									
20	(iii)	НҮРС	THET	CICAI	: NC											
	(iv)	TNA	-SEN	ISE:	ИО											
25	(vi)		ORC	SANIS	5M: F	Ratti				s Prot	P373	37 <b>7</b>				
30	(vii)		EDIAT				/nucl	lein	prot	tein						
	(xi)	SEQU	JENCI	E DES	SCRII	OITS	1: SI	EQ II	ои с	:5:						
3.5	Met 1	Asp	Val	Phe	Met 5	Lys	Gly	Leu	Ser	Lys 10	Ala	Lys	Glu	Gly	Val 15	Val
40	Ala	Ala	Ala	Glu 20	Lys	Thr	Lys	Gln	Gly 25	Val	Ala	Glu	Ala	Ala 30	Gly	Lys
	Thr	Lys	Glu 35	Gly	Val	Leu	Tyr	Val 40	Gly	Ser	Lys	Thr	Lys 45	Glu	Gly	Val
45	Val	His 50	Gly	Val	Thr	Thr	Val 55	Ala	Glu	Lys	Thr	Lys 60	Glu	Gln	Val	Thr
	Asn 65	Val	Gly	Gly	Ala	Val 70	Val	Thr	Gly	Val	Thr 75	Ala	Val	Ala	Gln	Lys 80
50	Thr	Val	Glu	Gly	Ala 85	Gly	Asn	Ile	Ala	Ala 90	Ala	Thr	Gly	Phe	Val 95	Lys
	Lys	Asp	Gln	Met	Gly	Lys	Gly	Glu	Glu 105	_	Tyr	Pro	Gln	Glu 110	Gly	Ile

Leu Glu Asp Met Pro Val Asp Pro Ser Ser Glu Ala Tyr Glu Met Pro 120 115 Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala 135 (2) INFORMATION FOR SEQ ID NO:6: (i) SEQUENCE CHARACTERISTICS: 10 (A) LENGTH: 134 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: not relevant (D) TOPOLOGY: not relevant 15 (ii) MOLECULE TYPE: peptide (iii) HYPOTHETICAL: NO (iv) ANTI-SENSE: NO 20 (vi) ORIGINAL SOURCE: (A) ORGANISM: Bos taurus (C) INDIVIDUAL ISOLATE: Swiss-Prot P33567 25 (vii) IMMEDIATE SOURCE: (B) CLONE: alpha synuclein protein 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6: Met Asp Val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly Val Val 35 Ala Ala Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys 25 Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val 35 40 Val Gln Gly Val Ala Ser Val Ala Glu Lys Thr Lys Glu Gln Ala Ser His Leu Gly Gly Ala Val Phe Ser Gly Ala Gly Asn Ile Ala Ala Ala 45 Thr Gly Leu Val Lys Lys Glu Glu Phe Pro Thr Asp Leu Lys Pro Glu Glu Val Ala Glu Glu Ala Ala Glu Glu Pro Leu Ile Glu Pro Leu Met 50 105 Glu Pro Glu Gly Glu Ser Tyr Glu Glu Gln Pro Gln Glu Glu Tyr Gln 115 120

Glu Tyr Glu Pro Glu Ala 130

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5	(2) INFOR	RMATI	ON F	OR S	SEQ I	D NC	):7:									
J	(i)		JENCE LEN TYF	IGTH :	142	ami	.no a		;							
10			STR						ant							
	(ii)	MOLE	ECULE	TYP	E: p	epti	de									
1 5	(iii)	НҮРС	THET	CICAL	.: NC	)										
15	(iv)	ANTI	- SEN	ISE:	ио											
20	(vi)	(A)	GINAI ORC INI	SANIS	SM: S	Serir				c L33	3860					
	(vii)		EDIAT				/nuc]	lein	homo	ologi	ıe					
25																
	(xi)	SEQU	JENCE	E DES	SCRIE	OIT	1: SI	EQ II	ОИО	:7:						
3 ()	Met 1	Asp	Val	Phe	Met 5	Lys	Gly	Leu	Ser	Lys 10	Ala	Lys	Glu	Val	Val 15	Ala
	Ala	Ala	Glu	Lys 20	Thr	Lys	Gln	Gly	Val 25	Ala	Glu	Ala	Ala	Gly 30	Lys	Thr
35	Lys	Glu	Gly 35	Val	Leu	Tyr	Val	Gly 40	Ser	Arg	Thr	Lys	Glu 45	Gly	Val	Val
·10	His	Gly 50	Val	Thr	Thr	Val	Ala 55	Glu	Lys	Thr	Lys	Glu 60	Gln	Val	Ser	Asn
-10	Val 65	Gly	Gly	Ala	Val	Val 70	Thr	Gly	Val	Thr	Ala 75	Val	Ala	Gln	Lys	Thr 80
45	Val	Glu	Gly	Ala	Gly 85	Asn	Ile	Ala	Ala	Ala 90	Thr	Gly	Leu	Val	Lys 95	Lys
	Asp	Gln	Leu	Ala 100	Lys	Gln	Asn	Glu	Glu 105	Gly	Phe	Leu	Gln	Glu 110	Gly	Met
50	Val	Asn	Asn 115	Thr	Gly	Ala	Ala	Val 120	Asp	Pro	Asp	Asn	Glu 125	Ala	Tyr	Glu
	Met	Pro		Glu	Glu	Glu	Tyr		Asp	Tyr	Glu	Pro	Glu	Ala		

	(2) INE	FORM	OITA	n FO	R SE	Q ID	NO:	8:									
5	(:	i) s	(B)	LENG TYPE STRA	TH: : am .NDEC	143 nino NESS	erist amin ació s: no ot re	no ac i ot re	eids eleva	int							
10			OLEC			_	eptic	ie									
	·		TOGY														
	·		NTI-														
15	(v	1) (		ORGA	MISI	M: T	orpe ISOL				ca rot :	P373	79				
20	(vi	i) [	IMMEI (B)					nucl	ein 1	homo	logu	e					
25			SEQUI														
	M [		Asp '	Val		Lys 5	Lys	Gly	Phe	Ser	Phe 10	Ala	Lys	Glu	Gly	Val 15	Val
30	I	Ala	Ala .		Glu 20	Lys	Thr	Lys	Gln	Gly 25	Val	Gln	Asp	Ala	Ala 30	Glu	Lys
	5	Thr	Lys	Gln 35	Gly	Val	Gln	Asp	Ala 40	Ala	Glu	Lys	Thr	Lys 45	Glu	Gly	Val
35	I	Met	Tyr 50	Val	Gly	Thr	Lys	Thr 55	Lys	Glu	Gly	Val	Val 60	Gln	Ser	Val	Asn
1.0		Thr 65	Val	Thr	Glu	Lys	Thr 70	Lys	Glu	Gln	Ala	Asn 75	Val	Val	Gly	Gly	Ala 80
40		Val	Val	Ala	Gly	Val 85	Asn	Thr	Val	Ala	Ser 90	Lys	Thr	Val	Glu	Gly 95	Val
45		Glu	Asn	Val	Ala 100	Ala	Ala	Ser	Gly	Val 105	Val	Lys	Leu	Asp	Glu 110	His	Gly
		Arg	Glu	Ile 115	Pro	Ala	Glu	Gln	Val 120	Ala	Glu	Gly	Lys	Gln 125		Thr	Gln
50		Glu	Pro 130	Leu	Val	Glu	Ala	Thr		Ala	Thr	Glu	Glu 140		Gly	Lys	

(2) INFORMATION FOR SEQ ID NO:9:

5	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 19 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
	<pre>(ii) MOLECULE TYPE: other nucleic acid      (A) DESCRIPTION: /desc = "primer #1F"</pre>	
10	(iii) HYPOTHETICAL: NO	
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:	
	ACGACAGTGT GTGTAAAGG	19
20	(2) INFORMATION FOR SEQ ID NO:10:	
20	<ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 20 base pairs</li><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: single</li></ul>	
25	(D) TOPOLOGY: linear	
	<pre>(ii) MOLECULE TYPE: other nucleic acid      (A) DESCRIPTION: /desc = "primer #13R"</pre>	
30	(iii) HYPOTHETICAL: NO	
35	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:	
	AACATCTGTC AGCAGATCTC	20
	(2) INFORMATION FOR SEQ ID NO:11	
40	(i) SEQUENCE CHARACTERISITCS	
	(A)LENGTH: 2809 base pairs	
	(B) TYPE: NUCLEIC ACID	
	(C)STRANDEDNESS: DOUBLE	
	(D) TOPOLOGY: LINEAR	
45	(ii) MOLECULAR TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	

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BNSDCXCIE <WC 9859050A1 + >

(iv) ANTI-SENSE: NO

#### (v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 139A20 HUMAN BETA SYNULEIN GENE

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCGCCGCAGC CGCCGCTCCA TCCCCAGCCC CGGCCCCGCA TCCGGTTTGG AAGGGGGGCTG 5 CAAGTTTGCA AGGGGCCCGG GANAAAAANC GAGCAGTGGC CCTTCCCGCG TCCCCAGGGT TTCAAGGGAC GCTAGGANTN TCCGCGGCCC TGGAGGTTCG CACTGGGGAG TGGGGTGAGA TGGGGGGAAA GCGGGAGGGG GCTCAGGGTC CAGAAGGGCN CCGCGGTCTC GGGAGTAGGG GGGCATNTGC GTCCCGCGGG AGGGGCTGGG GTGAGAGTGC GGGGCCAGTG CACCGGTGCC CGTGTATCGC CCTCCCCAGG CCGCCAGGAT GGACGTGTTC ATGAAGGGCC TGTCCATGGC 10 CAAGGAGGC GTTGTGGCAG CCGCGGAGAA AACCAAGCAG GGGGTCACCG AGGCGGCGGA GAAGACCAAG GAGGGCGTCC TCTACGTCGG TGGGCNGGGG GCNGGGTTTC TGGGGCTGCA GGGCTGGGGG TCCCCCTACA GTGTGGAGCT GGGGCCGGGT CCCGGGGAGG GGGGTTCTGG GCAAGATAAT ATNANTCAGC AGATGGGGCN AGGTCANCAN GGGTCATAAG GGACATACCC ANCCCATAGA ANCCTGGGTC TGTATCCGGA AATGGGGACA CGGGGCGGGC TGATGAGGTG 15 GGGGGCTCCA NCTGAAAGGC CAGGGACCAN TGCANTNATA AAANCACACA NCCTCCTTTT TCTTATCTTT TTTACCATTA TTAATAGTTA TCTGGTGTTG AACACTTTCT GTATGCCAAG TACTGGGTAA AATGTCATAA CATCCATTTC CTCATGTAAT GCTTCCGCCC ATTCTACAGG TAAGGGAAAC TGGGCTTCCC ATTGGTAGNT AAATTTTAGG TTCAGAAAGG CTTGAATTGA ATGTCAGTTC AGCCAATTTC TTAGTGGTGG AACCAAACTG AGTTCCATCC GTGAAACGGG 20 GACAATAACA GCACCCGCTT CCCAGGGCTG GGGAAAAGTG AAGTGCAGCG GGGCAGGCAG AGGACTTGAC ACAGCACTGG CCCTCAGCCA ACATCCACTA GAGGGGTGGG GTATCGCATC AGGTGGGAGA GAACTGCAAC CCTTGCAGAC AGAGGTGTGG GGCCCAGTGC AGTGATAAGA CGGGGGTTAA CATGGGGGTG CAGGTTGTAG GATNTGGGGA CCCAAGGAGG CAGTGACGGG GCCAGGATGC CCACTCTGTA ATCACCATGC TGTGCTGGAG TTTCTGTTCC CTCAGCGCAG 25 AGTCCTTAAA TGTGCCGCTT TTTCTNCCCT GCAGGAAGCA AGACCCGAGA AGGTGTGGTA CAAGGTGTGG CTTCAGGTAC TAGCCCAGCC CTGGCACCAG CCCTTCTCTC AMTTAGGCGG

ATGATCTGGC CGGGAACCAG AGGGCGGGGG CGGGGGAGAC TCCCAAGGCT TCTGCGGGAA TGCTCCGTGG GGAGGGCAGG CCCTGGGATA CTACAAGGCA GGGCATCGGT GTTTCCCCCT GGCTCCCAAA CCCCTTCCTC AACCCCCTCC CTGCTCCAGT GGCTGAAAAA ACCAAGGAAC AGGCCTCACA TCTGGGAGGA GCTGTGTTCT CTGGGGCAGG GAACATCGCA GCAGCCACAG GACTGGTGAA GAGGGAGGAA TTCCCTACTG ATCTGAAGGT AAGCGATCCT TCTGACCCGC ACATGCAGGC AAACACACAC ACACACACA ACACACAC ACACACACA GGCACACAAA TAAACCTGTC ACCATCCCCG CCCCCTAAT CCTGCCACCA GCTTGGAACA CAAGCCACTT TGCCTCCCAT CCTGCNGGCC CGTGCTAGAC TCAGCTCAGA ATGCATCTGA ATAANGGCGT GCATGGGTGT GACGCTCCCG GTGATGGGGA CCCAGACCTG GCTGTCTGCG TGTATCCTGC TTGCCAGCGT GACCCATATG ACTTCTGGCC ACGTCTGCAT GTGTCAATGA TTGTTCATTC ATTTCTTTTC ATTCAACAAA TATCCATGCC ANANCCAGCC CTGTCCTTGA GCTTCCAGNT CCCTTTCAGC CNAGGGGAGC NTGAGGGTTA TTTTTGGGGT CCCGATGCCC AGCACAGAGC CTGACACAAA GGATGAGGCA TAAGCTGGTG ANTGAGTATC CAAATGGTGG AAGTGTGGAG GNTGCCAGGC ATTGGGGGAG CGGCGTGGAG AGCCAGCTCC CCAATCCATG CTGCCACTTC AACTGTGATT CGGGGGAATT TCCCCCTTCA CCTCCATCCC &CTTCCAAGG CACTCCAAAT AAATAACTGA ATTAGAAATT ATCCTTGTTT TGCCAACCCA CCCTAGCCTT CCCCACTCCA ACCCACCCAA AGCTTACCAC TGTGGGAATT TGGGGGGCAT CCTGGCTGTC CTCACGAGTC CTGACCTTTT CTGCCCACAG CCAGAGGAAG TGGCCCAGGA AGCTGCTGAA GAACCACTGA TTGAGCCCCT GATGGAGCCA GAAGGGGAGA GTTATGAGGA CCCACCCCAG GAGGAATATC AGGAGTATGA GCCAGAGGCG TAGGGGCCCA GGAGAGCCCC CACCAGCAGC ACAATTCTGT CCCTGTCCCT GCCCCGCCC CCAGAGCCAG GGCTGTCCTT AGACTCCTTC TCCCCAATCA CGAGATCTTC CTTCCGCTCT GAGGCAACCC CCTCGGAGCC TGTGTTAGTG TCTGTCCATC TGTCTGTCCT ACCCGCCGC GTCCAACCCC GGGGCATGGA CAGGGCCAGG GTTGCGGTCG CGGCTGGGAG CCTCGCCCCT CCAGTGTTGC CTCCTCCCAT CCAGCGTCTG CGCG

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# (2) INFORMATION FOR SEQ 1D NO:12

#### (i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 223 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

5 (ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 174P13 HUMAN GAMMA SYNULEIN GENE, 5' END

10 (vi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGGGAGATCC AGCTCCGTCC TGCCTGCAGC AGCACACCC TGCACACCCA CCATGGATGT

CTTCAAGAAG GGCTTCTCCA TCGCCAAGGA GGGNGTGGTG GGTGCGGTGG AAAAGACCAA

GCAGGGGGTG ACGGAAGCAG CTGAGAAGAC CAAGGAGGGG GTCATGTATG TGGGATTACA

TTTTTTTTT AAAGAAAGAA TAAATTAATT GTGATTAAAG TTG

15

- (2) INFORMATION FOR SEQ ID NO:13
  - (i) SEQUENCE CHARACTERISITCS
    - (A) LENGTH: 677
    - (B) TYPENUCLEIC ACID
- 20 (C) STRANDEDNESS: DOUBLE
  - (D) TOPOLOGY: LINEAR
  - (ii) MOLECULAR TYPE: DNA (genomic)
  - (iii) HYPOTHETICAL: NO
  - (iv) ANTI-SENSE: NO
- (v) IMMEDIATE SOURCE:
  - (A) CLONE: BAC clone 174Pl3 HUMAN GAMMA SYNULEIN GENE, 3' END
  - (vi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTTTTTNAGG GGGGAAAACA GGGAATANAA AAANANGGGG GGGGGTTTTT NNGGGGGGGG
GGGGAAAANG GTTNGGGGN NAACCNAAAN AAANNCCNAN GGGGGGGNN ANTNAANTTT
TGGGAACCCA AAGCCCNAGG AGGATTTTTN GTNAANAACG TNACCTCNAG TGGGNCGAGG
AAGACCAAGG AAANGCCCAA CNCGGTTGAN CGAGGCTGTG GTGAACANCG TNCAACNCTG
TGCCCNCCAA NANCGTGGAG GNGGCGGAGA ACATCSCGGT CACCTCCGGG GTGGTGCGCM
AGGAGGACTT GAGGCCATCT KCCCCCCMAC AGGAGGGTGT GGCATCCMAA GARAAAGAGG
AAGTGGCAGA GGAGGCCCAG AGTGGGGGAR ACTCACGGC TACAGGCCAG CGTGGATGAC
CTGAAGAGGG CTCCTCTGCC TTGGACACCA TCCCCTCCTA GCACAAGGAG TGCCCGCCTT
GAGTGACATG CGGCTGCCCA CGCTCTGCC CTCGTCTTCC TGGCCACCCT TGGCCTGTCC
ACCTGTGCTG CTGCACCAAC CTCACTGCC TCCCTCGGC CCACCCACCC TCTGGTCCTT
CTGACCCCAC TTATGCTGCT GTGAATTTTT TTTTTAAATG ATTCCAAATA AAACAAAA

15 (2) INFORMATION FOR SEQ ID NO:14

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BNSC/IXID +WC

9859050**A**1

- (i) SEQUENCE CHARACTERISITCS
  - (A) LENGTH: 1181 base pairs
  - (B) TYPE: NUCLEIC ACID
  - (C) STRANDEDNESS: DOUBLE
- 20 (D) TOPOLOGY: LINEAR
  - (ii) MOLECULAR TYPE: DNA (genomic)
  - (iii) HYPOTHETICAL: NO
  - (iv) ANTI-SENSE: NO
  - (vii) IMMEDIATE SOURCE:
- 25 (A)CLONE: human alpha synuclein gene/ exons 1 and 2 plus flanking intron sequences
  - (viii) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: 4
- (B) MAP POSITION: 4q21-q22
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AATTTCAGCG ATGCGAGGGC AAAGCGCTCT CGGCGGTGCG GTGTGAGCCA CCTCCCGGCG 5 CTGCCTGTCT CCTCCAGCAG CTCCCCAAGG GATAGGCTCT GCCCTTGGTG GTCGACCCTC AGGCCCTCGN TCTCCCAGGN CGACTCTGAC GAGGGGTAGG GGGTGGTCCC CNGGAGGACC CAGAGGAAAG GCNGGGACAA GAAGGGAGGG GAAGGGGAAA GAGGAAGAGG CATCATCCCT AGCCCAACCG CTCCCGATCT CCACAAGAGT GCTCGTGACC CTAAACTTAA CGTGAGGCGC AAAAGCGCCC CAACCTTTTC CCGCCTTGNN CCAGGCAGGC GGCTGGAGTT GATGGCTCAC 10 CCCGCGCCCC CTGCCCCATC CCCATCCGAG ATAGGGACGA GGAGCACGCT GCAGGGAAAG CAGCGAGCGC CGGGAGAGGG GCGGGCAGAA GCGCTGACAA ATCAGCGGTG GGGGCGGAGA GCCGAGGAGA AGGAGAAGGA GGAGGACTAG GAGGAGGAGG ACGGCGACGA CCAGAAGGGG CCCAAGAGAG GGGGCGAGCG ACCGAGCGCC GCGACGCGAA GTGAGGTGCG TGCGGGCTCA GCGCAGACCC CGGCCCGGCC CCTCCTGAGA GCGTCCTGGG CGCTCCCTCA CGCCTTGCCT 15 TCAAGCCTTC TGCCTTTCCA CCCTCGTGAG CGGAGAACTG GGAGTGGCCA TTCGACGACA GGTTAGCGGG TTTGCCTCCC ACTCCCCCAG CCTCGCGTCG CCGGCTCACA GCGGCCTCCT CTGGGGACAG TCCCCCCGG GTGCCCCTCC GCCCTTCCTG TGCGCTCCTT TTCCTTCTTC GNGGAGGAGT CGGAGTTGTG GAGAAGCAGA GGGACTCAGG TAAGTACCTG TGGATCTAAA CGGGNGTCTT TTGGAAATCC TGGAGAACGC CGGATGGAGA CGAATGGTCG TGGGNACCGG 20 GAGGGGGTGG TGCTGCCATG AGGACCGCTG GGCCAGGTCT CTGGGAGGTG AGTACTTGTC CTTTGGGGAG CTAAGGAAAG AGACTTGACC TGGCTTTCGT CCTGCTTCTG ATATTCCCTT CTCCACAAGG GCTGAGAGNT TAGGCTGCTT CTCCGGGATC C

- 25 (2) INFORMATION FOR SEQ ID NO:15
  - (i) SEQUENCE CHARACTERISITCS
    - (A) LENGTH: 536 base pairs

- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR
- (ii) MOLECULAR TYPE: DNA (genomic)
- 5 (iii) HYPOTHETICAL: NO
  - (iv) ANTI-SENSE: NO
  - (vii) IMMEDIATE SOURCE:
  - (A) CLONE: human alpha synuclein gene/ exon 3 plus flanking intron sequences
- 10 (viii) POSITION IN GENOME:

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BNSDCXCLC RWT: 9859050A\* or

- (A) CHROMOSOME/SEGMENT: 4
- (B) MAP POSITION: 4q21-q22
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
- CTTAAAAGAG TCTCACACTT TGGAGGGTTT CTCATGATTT TTCAGTGTTT TTTGTTTATT
  TTTCCCCGAA AGTTCTCATT CAAAGTGTAT TTTATGTTTT CCAGTGTGG GTAAAGAAAT
  TCATTAGCCA TGGATGTATT CATGAAAGGA CTTTCAAAGG CCAAGGAGGG AGTTGTGGCT
  GCTGCTGAGA AAACCAAACA GGGTGTGGCA CAAGCAGCAG GAAAGACAAA AGAGGGTG.T
  CTCTATGTAG GTAGGTAAAC CCCAAATGTC AGTTTGGTGC TTGTTCATGA GTGATGGGTT
  AGGATAACAA TACTCTAAAT GCTGGTAGTT CTCTCTTG ATTCATTTT GCATCATTGC
  TTGTCAAAAA GGTGGACTGA GTCAGAGGTA TGTGTAGGTA GGTGAATGTG AACGTGTGTA
  TNTGAGCTAA TAGTAAAAAAT GCGACTGTTT GCTTTCAGA TTTTTAATTT TGCCTAATAT
  NTATGACTTN TTAAAATGAA TGTTTCTGTA CTACATAATT CTATNTCAGA GACAGT
- (2) INFORMATION FOR SEQ ID NO:16
- 25 (i) SEQUENCE CHARACTERISITCS
  - (A) LENGTH: 650 base pairs
  - (B) TYPE: NUCLEIC ACID

- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR
- (ii) MOLECULAR TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- 5 (iv) ANTI-SENSE: NO

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DNODOO 5 JAKE

- (vii) IMMEDIATE SOURCE:
- (A)CLONE: human alpha synuclein gene/ exon 4 plus flanking intron sequences
  - (viii) POSITION IN GENOME:
- 10 (A) CHROMOSOME/SEGMENT: 4
  - (B) MAP POSITION: 4q21-q22
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGCAGGTCA ACGGATCTGT CTCTAGTGCT GTACTTTTAA AGCTTCTACA GTTCTGAATT

CAAAATTATC TTCTCACTGG GCCCCGGTGT TATCTCATTC TTTTTCTCC TCTGTAAGTT

GACATGTGAT GTGGGAACAA AGGGGATAAA GTCATTATTT TGTGCTAAAA TCGTAATTGG

AGAGGACCTC CTGTTAGCTG GGCTTTCTTC TATNTATTGT GGTGGTTAGG AGTTCCTTCT

TCTAGTTTTA GGATATATA ATATATTTTT TCTTTCCCTG AAGATATAAT AATATATAA

CTTCTGAAGA TTGAGATTTT TAAATTAGTT GTATTGAAAA CTAGCTAATC AGCAATTTAA

GGCTAGCTTG AGACTTATGT CTTGAATTTG TTTTTGTAGG CTCCAAAACC AAGGAGGAG

TGGTGCATGG TGTGGCAACA GGTAAGCTCC ATTGTGCTTA TAAAAAAAAA

GTATCTAGTG ATTAGTGTGG CCCAGTATCA AGATTCCTAT TGAAATTGTA AAACAATCAC

TGAGCATCTA AGAACATATC AGTCTTATTG AAACTGAATT CTTTATAAAG TATTTTTAAA

TAGGTAAAATA TTGATTATAA ATAAAAAATA TACTTGCCAA GAATAATGAG

- 25 (2) INFORMATION FOR SEQ ID NO:17
  - (i) SEOUENCE CHARACTERISITCS
    - (A) LENGTH: 504 base pairs

- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR
- (ii) MOLECULAR TYPE: DNA (genomic)
- 5 (iii) HYPOTHETICAL: NO
  - (iv) ANTI-SENSE: NO
  - (vii) IMMEDIATE SOURCE:
  - (A)CLONE: human alpha synuclein gene/ exon 5 plus flanking intron sequences
- 10 (viii) POSITION IN GENOME:
  - (A) CHROMOSOME/SEGMENT: 4
  - (B) MAP POSITION: 4q21-q22
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
- ATATCTTAGC CAAGATTCAA TGTTTGGTTG AACCACACTC ACTTGACATC TTGGTGGCTT

  TTGTTTCTTC TGACCACTCA GTTATCTATG GCATGTGTAG ATACAGGTGT ATGGAANCGA
  TGGCTAGTGG AAGTGGAATG ATTTTAAGTC ACTGTTATTC TACCACCCTT TAATCTGTTG
  TTGCTCTTTA TTTGTACCAG TGGCTGAGAA GACCAAAGAG CAAGTGACAA ATGTTGGAGG
  AGCAGTGGTG ACGGGTGTGA CAGCAGTAGC CCAGAAGACA GTGGAGGGAG CAGGGAGCAT
  TGCAGCAGCC ACTGGCTTTG TCAAAAAAGGA CCAGTTGGGC AAGGTATGGC TGTGTACGTT

  20 TTGTGTTACA TTTATAAGCT GGTGAGATTA CGGTTCATTT TCATGTGAAG CCTGGAGGCA
  GGAGCAAGAT ACTTACTGTG GGGAACGGCT ACCTGACCCT CCCCTTGTGA AAAAGTGCTA
  CCTTTATATT GGTCTTGCTT GTTT
  - (2) INFORMATION FOR SEQ ID NO:18
- 25 (i) SEQUENCE CHARACTERISITCS

BNSDGGER < W.D. 9859050A1 L. > ...

- (A) LENGTH: 727 base pairs
- (B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv)ANTI-SENSE: NO

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DNISE OVER LANCE GUEGOSOMA

GCTGTCT

(vii) IMMEDIATE SOURCE:

(A)CLONE: human alpha synuclein gene/ exons 1 and 2 plus flanking intron sequences

(viii) POSITION IN GENOME:

10 (A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

(2) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 1596 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

5 (D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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BNSDOOR

(vii) IMMEDIATE SOURCE:

10 (A)CLONE: human alpha synuclein gene/ exon 7 plus flanking intron sequences

(viii) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: 4
- (B) MAP POSITION: 4q21-q22
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTTTGATTT TCTAATATTA GGAAGGGTAT CAAGACTACG AACCTGAAGC CTAAGAAATA

TCTTTGCTCC CAGTTCTTG AGATCTGCTG ACAGATGTTC CATCCTGTAC AAGTGCTCAG

TTCCAATGTG CCCAGTCATG ACATTTCTCA AAGTTTTTAC AGTGTATCTC GAAGTCTTCC

ATCAGCAGTG ATTGAAGCAT CTGTACCTGC CCCCACTCAG CATTTCGGTG CTTCCCTTTC

ACTGAAGTGA ATACATGGTA GCAGGGTCTT TGTGTGCTGT GGATTTTGTG GCTTCAATCT

ACGATGTTAA AACAAATTAA AAACACCTAA GTGACTACCA CTTATTTCTA AATCCTCACT

ATTTTTTTGT TGCTGTTGTT CAGAAGTTGT TAGTGATTTG CTATCATATA TTATNAGATT

TTTAGGTGTC TTTTAATGAT ACTGTCTAAG AATAATGACG TATTGTGAAA TTTGTTAATA

TATATNATAC TTAAAAAATAT GTGAGCATGA AACTATGCAC CTATAATACT AAATATGAAA

TTTTACCATT TTGCGATGTG TTTTATTCAC TTGTGTTTGT ATATNAATGG TGAGAATTAA

AATAAAACGT TATCTCATTG CAAAAATATT TTATTTTTAT CCCATCTCAC TTTAATAAGT

### WHAT IS CLAIMED IS:

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1. An isolated nucleic acid comprising a nucleotide sequence encoding a mutated human synuclein protein or homologue thereof.

- 2. The isolated nucleic acid of claim 1 wherein said mutated synuclein protein is selected from the group consisting of alpha, beta and gamma synuclein proteins.
- 3. The isolated nucleic acid of claim 2 wherein said mutated30 synuclein protein is the alpha synuclein protein.
  - 4. The isolated nucleic acid of claim 3 wherein said nucleotide sequence contains at least one mutation at base pair position 209.
- The isolated nucleic acid of claim 4 wherein said mutation at position 209 is a change from guanine to adenine.
  - 6. The isolated nucleic acid of claim 5 having the sequence given in SEQ ID NO. 1.
  - 7. An oligonucleotide complementary to a portion of the synuclein gene, wherein said portion comprises a mutation associated with predisposition to Parkinson's Disease.
- 45 8. The oligonucleotide of claim 7 wherein said mutation is at base pair position 209 in the synnuclein gene.

9. The oligonucleotide of claim 8 wherein said mutation is a change from guanine to adenine.

10. A vector comprising the isolated nucleic acid of claim 1.

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- 11. A host cell comprising the vector of claim 10.
- 12. A method of affecting characteristics of Parkinson's Disease, comprising expressing nucleic acids which are implicated in disease development in cultured cells through the use of expression vectors.
  - 13. The method of claim 12 wherein the said nucleic acid is selected from the group consisting of alpha, beta, and gamma synuclein genes.
- 14. The method of claim 13 wherein the said nucleic acid encodes the mutated alpha synuclein protein.
  - 15. The method in claim 14 wherein the said mutated alpha synuclein protein contains at least one mutation at base pair 209.
- 20 16. The method of claim 15 wherein said mutation at position 209 is a change from quanine to adenine.
  - 17. An isolated human synuclein protein or peptide containing at least one mutation.

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18. The isolated human synuclein protein or peptide of claim 17 wherein said protein or peptide is selected from the group consisting of

the human alpha, beta and gamma synuclein proteins or fragments thereof.

19. The isolated human synuclein protein or peptide of claim 18 having the sequence given in SEQ ID NO 5.

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20. The isolated human synuclein protein or peptide of claim 19 wherein said protein or peptide is the alpha synuclein gene or a fragment thereof.

- 10 21. The isolated protein or peptide of claim 20, wherein said mutation is at amino acid position 53.
  - 22. The isolated protein or peptide of claim 21, wherein said mutation is an alanine to threonine substitution.

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23. An antibody specific for the protein or peptide of claim 17.

24. A method of detecting subjects at increased risk for Parkinson's Disease, comprising:

obtaining a sample comprising nucleic acids, proteins or tissues from the subjects, and

detecting in the nucleic acids, proteins or tissues the presence of a mutation which is associated with Parkinson's disease,

thereby identifying subjects at increased risk for the disease.

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25. The method of claim 24 wherein said mutation is located on human chromosome four.

26. The method of claim 25 wherein said mutation is located in the alpha synuclein gene.

- 27. The method of claim 26 wherein said mutation causes an amino acid substitution at position 53.
  - 28. The method of claim 27 wherein said mutation causes an alanine to threonine substitution at position 53.
- 29. The method of claim 24 wherein said detecting step comprises combining a nucleotide probe which selectively hybridizes to a nucleic acid containing said mutation, and detecting the presence of hybridization.
- 30. The method of claim 29 wherein said nucleotide probe is an oligonucleotide complementary to a portion of the synuclein gene, wherein said portion comprises a mutation associated with predisposition to Parkinson's Disease.
- 20 31. The method of claim 30 wherin the mutation of said oligonucleotide is at base pair position 209 in the alpha synuclein gene.
  - 32. The method of claim 31 wherein the mutation of said oligonucleotide is a change from guanine to adenine.

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33. The method of claim 24 wherein said detecting step comprises amplifying a nucleic acid product comprising said mutation, and detecting

the presence of said mutation in the amplified product.

34. The method of claim 33 wherein said detecting step comprises selectively amplifying a nucleic acid product comprising said mutation, and detecting the presence of amplification.

35. The method of claim 34 wherein said amplifying step comprises at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids.

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- 36. The method of claim 35 wherein said amplifying step uses two oligonucleotides.
- 37. The method of claim 36 wherein said two oligonucleotides have the sequences of SEQ ID NOs 2 and 3.
  - 38. The method of claim 24 wherein said detecting step comprises detecting the presence or absence of a restriction endonuclease site as detected by enzymatic digest of said sample of nucleic acids.

- 39. The method of claim 38 wherein said restriction endonuclease site is recognized by *Tsp*451.
- 40. The method of claim 24 wherein said detecting step comprises chain termination with a labeled dideoxynucleotide.
  - 41. An oligonucleotide complementary to a nucleic acid sequence

which flanks a mutation in the synuclein gene that is associated with predisposition to Parkinson's disease, wherein said oligonucleotide may be used in diagnostic screens in the amplification of a nucleic acid sequence comprising said mutation.

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- $$42\,.$$  The oligonucleotide of claim 41 having the sequence of SEQ ID NO 2.
- \$43.\$ The oligonucleotide of claim 41 having the sequence of SEQ ID  $$10^{\circ}$$  NO 3.
  - 44. The method of claim 24 wherein said detection step comprises identification of said mutations with an antibody.
- 15 45. The method of claim 44 wherein said antibody is directed against an isolated human synuclein protein or peptide containing at least one mutation.
- 46. The method of claim 45 wherein said isolated human synuclein protein or peptide is selected from a group consisting of the human alpha, beta, and gamma synuclein proteins or fragments thereof.
  - 47. The method of claim 46 wherein said isolated human synuclein protein or peptide has the mutated sequence given in SEQ ID NO 5.

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48. The method of claim 47 wherein said mutation is at amino acid position 53.

49. The method of claim 48 wherein said mutation is an alanine to threonine substition

50. A diagnostic kit comprising the oligonucleotide of claim 41.

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- 51. A diagnostic kit comprising the oligonucleotide of claim 42.
- 52. A diagnostic kit comprising the oligonucleotide of claim 43.
- 10 53. A diagnostic kit comprising the oligonucleotide of claim 7.
  - 54. A diagnostic kit comprising the oligonucleotide of claim 8.
  - 55. A diagnostic kit comprising the oligonucleotide of claim 9.

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- 56. A diagnostic kit comprising the antibody of claim 23.
- 57. An isolated nucleic acid comprising a mutation in a human synuclein gene or homologue thereof.

- 58. The isolated nucleic acid of claim 57 wherein said synuclein gene is the alpha synuclein gene.
- 59. The isolated nucleic acid of claim 58 wherein said mutation occurs at base pair position 209.
  - 60. The isolated nucleic acid of claim 59 wherein said mutation is

a change from guanine to adenine.

61. The isolated nucleic acid of claim 60 having the sequence given in SEQ ID NO 1.

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- 62. A transgenic animal which expresses a mutated synuclein protein, wherein said animal may be used as an animal model for Parkinson's disease.
- 10 63. The transgenic animal of claim 62, wherein said mutated synuclein protein is an alpha synuclein protein.
  - 64. A method of screening a compound for the ability to reverse the self-aggregation of synuclein proteins, comprising exposing an aggregate of synuclein proteins to a test compound and observing whether or not the aggregate is dissolved.
  - 65. The method of claim 64 wherein said test compound is a synuclein peptide.

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- 66. The method of claim 65 wherein said peptide comprises a mutation.
- 67. The method of claim 64 wherein said test compound is an antibody.
  - 68. The method of claim 64, wherein said observing step comprises

Congo red staining, electron microscopy or CD spectrometry.

69. The method of claim 64 wherein said protein aggregate is located within an animal.

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- 70. A method of screening a compound for the ability to inhibit the self-aggregation of synuclein proteins, comprising exposing a population of synuclein proteins to a test compound under conditions which promote self-aggregation in the absence of said compound and observing whether or not self-aggregation of said proteins is inhibited.
- 71. The method of claim 70 wherein said test compound is a synuclein peptide.
- 72. The method of claim 71 wherein said peptide comprises a mutation.
  - 73. The method of claim 70 wherein said test compound is an antibody.

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74. The invention substantially as disclosed and described.

Figure 1

getaateageaatttaaggetaggaettatgtettgaatttgtttltgtagGCICCAAAACCAAGGAGGGGGGGGTGCATGGIGTGAACAGGtaageteeattg Gly Ser Lys Thr Lys Glu Gly Val Val His Gly Val (Thr) Thr က

2/16

Figure 2

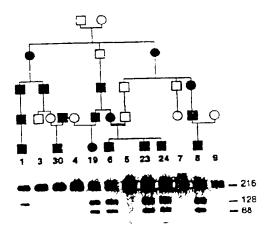


Figure 3

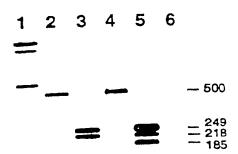
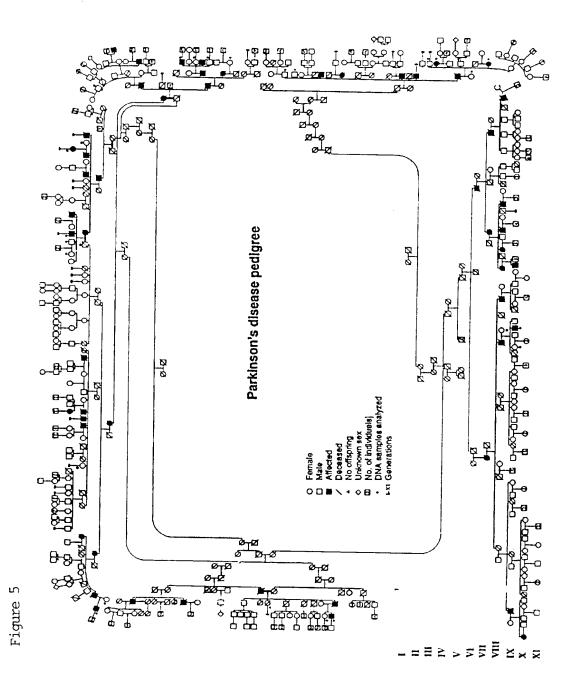


Figure 4

Homo sapiens Rattus norvegicus Bos taurus Serinus canaria Torpedo californica	Horno sapiens Rattus norvegicus Bos laurus Serinus canara Torpedo californica	Homo sapiens Rattus norvegicus Bos taurus Serinus canaria Torpedo californica	Homo sapiens Rattus norvegicus Bos taurus Serinus canarta Torpedo californica
10 1	40 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	110 110 110 110 110 110 120 120 120 130 1410 1420 1420 1420 1430 1430 1430 1430 1430 1430 1430 143	130 140 1
			•



BNSE/CRIE +: WO - 9859050A1 + >

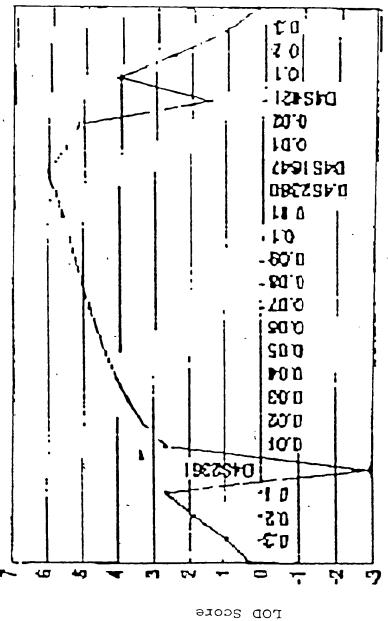


Figure 6

109979	clone	5'	31	gene
111098   T83410   T81593   Alpha   111090   T83411   T81593   Alpha   111090   T83411   T81593   Alpha   135534   R31354   R32856   Alpha   135534   R31354   R32856   Alpha   141246   R66863   R67383   Alpha   141246   Alpha   R66863   R67383   Alpha   R67384   R68914   R6885   Alpha   R68914   R66869   Alpha   R68914   R6861   R69814   R68914   R69914   R68914   R69914			T88834	
111090   T83411   T81593   alpha     130048   R11619   (R19409)   alpha     135534   R31354   R32856   alpha     141246   R66663   R67383   alpha     145594   R78091   R77746   alpha     171906   H19290   H19291   beta     172284   H19556   H19474   beta     172284   H19556   H19474   beta     172749   H19685   beta     173546   H41126   beta     173174   H47503   H47504   alpha     1210768   H68914   H68869   alpha     213616   H70324   H70325   alpha     213616   H70324   H70325   alpha     238027   H62070   alpha     248153   N53829   N73325   alpha     24991   (T80528)   R39000   alpha     26298   R13508   (R20629)   alpha     266817   N2861   N21457   alpha     266628   N22757   alpha     27342   R37173   alpha     280344   (N50305)   N47094   alpha     2904142   N68597   alpha     2904142   N68597   alpha     2904142   N68597   alpha     307767   W21278   alpha     3040635   W56712   W56757   alpha     340635   W56712   W56577   alpha     340636   W55986   W56276   alpha     346647   W94390   W74638   alpha     346766   W7685   W7078   alpha     34683   W55986   W56276   alpha     346647   W94390   W74638   alpha     346647   W94390   W74638   alpha     346647   W94390   W74638   alpha     346647   W94390   W74638   alpha     346766   W7685   W76784   alpha     34683   AA010546   AA010547   alpha     34683   AA022809   AA022690   alpha     34683   AA02869   AA026608   alpha     359349   AA010546   AA010547   alpha     364632   AA022809   AA022690   alpha     364632   AA022809   AA022690   alpha     366473   R16018   R16119   alpha     48607   H10267   H10213   alpha     49611   H29080   H26976   alpha     66473   R16018   R16119   alpha     667794   AA258686   AA258608   alpha     69907   T48654   T48655   alpha     69907   T48654   F03651   alpha     667794   AA258686   F03651   alpha     69907   T48654   F03651   alpha     69007   T48654   F03651   alpha     69007   T48654   F03651   alpha     69008   F03254   F06891   alpha     60008   F03254   F068961   alpha     60008   F03254   F068961   alpha				
130048			T81593	
135534 R31354 R32856 alpha 141246 R66863 R67383 alpha 145594 R78091 R77746 alpha 171906 H19290 H19291 beta 171906 H19290 H19291 beta 172284 H19556 H19474 beta 172749 H19685 beta 175546 H41126 beta 173174 H47503 H47504 alpha 210768 H68914 H66889 alpha 213616 H70324 H70325 alpha 213616 H70324 H70325 alpha 236027 H62070 alpha 248153 N53829 N73325 alpha 24991 (T80528) R39000 alpha 26298 R13508 (R20629) alpha 26298 R13508 (R20629) alpha 266828 N22757 alpha 27342 R37173 alpha 2908344 (N50305) N47094 alpha 290894 N72005 alpha 307767 W21278 Alpha 340635 W56712 W56757 alpha 340683 W55986 W56276 alpha 346647 W94390 W74638 alpha 346647 W94390 W74638 alpha 346796 W79585 W79784 alpha 346796 W79585 W79784 alpha 346796 W79585 W79784 alpha 346796 H70326 H00306 H00				
141248 R66663 R67383 elpha 145594 R78091 R77746 alpha 171906 H19290 H19291 beta 172284 H19556 H19474 beta 172749 H19685 beta 175546 H41126 beta 193174 H47503 H47504 alpha 210768 H66914 H66869 alpha 213616 H70324 H70325 alpha 236027 H62070 alpha 236027 H62070 alpha 248153 N53829 N73325 alpha 24991 (T80528) R39000 alpha 26298 R13508 (R20629) alpha 26298 R13508 (R20629) alpha 2626817 N28661 N21457 alpha 280644 (N50305) N47094 alpha 290894 N72005 alpha 2904142 N88597 alpha 340635 W56712 W56757 alpha 340683 W55986 W56278 alpha 346647 W94390 W74638 alpha 346766 W76885 W78784 alpha 346766 W76885 W78784 alpha 346766 W76885 W78784 alpha 346764 R56327 R56245 alpha 346764 R56327 R56245 alpha 34911 H29080 H28976 alpha 48607 H10267 H10213 alpha 49811 H29080 H28976 alpha 66473 R16018 R16119 alpha 667794 AA258686 AA258608 alpha 66907 T48654 T48655 alpha 667794 AA258686 AA258608 alpha 6697794 AA258686 AA258608 alpha 667794 AA258686 F111691 beta 667794 AA258686 F116811 beta 667794 AA258686 F111691 alpha 667794 AA258686 F111691 alpha 667794 AA258686 F111691 beta 667794 AA258686 F111691 alpha 667794 AA258686 F11691 alpha 667794 AA258686 F111691 alpha 667794 AA36791 F07521 alpha 667794 AA36791 F07521 alpha 667794 AA36791 F07521 alph				
145594 R78091 R77746 alpha 171906 H19290 H19291 beta 171906 H19290 H19291 beta 172284 H19556 H19474 beta 172749 H19685 beta 172749 H19685 beta 173546 H41126 beta 193174 H47503 H47504 alpha 210768 H66914 H66869 alpha 213616 H70324 H70325 alpha 236027 H62070 alpha 236027 H62070 alpha 248153 N53829 N73325 alpha 24991 (180528) R39000 alpha 26298 R13508 (R20629) alpha 265817 N28661 N21457 alpha 266628 N22757 alpha 27342 R37173 alpha 280344 (N50305) N47094 alpha 290894 N72005 alpha 290894 N72005 alpha 307767 W21278 alpha 340683 W55912 N56757 alpha 340683 W55988 W56276 alpha 346647 W94390 W74638 alpha 346796 W70685 W70784 alpha 359349 AA010546 AA010547 alpha 364832 AA022809 AA022690 alpha 39915 R50355 beta 40764 R56327 R56245 alpha 364807 H10267 H10213 alpha 49811 H29080 H28976 alpha 50202 H17862 beta 667794 AA258686 AA258608 alpha 69907 T48654 T48655 alpha 667794 AA258686 AA258608 alpha 739009 AA421586 beta 739014 (AA42185) AA421667 beta 771903 AA443803 alpha 667794 AA258696 AA258608 alpha 69907 T48654 T48655 alpha 667794 AA258696 AA258608 alpha 69907 T48654 T48655 alpha 667794 AA36097 AA293803 gamma 671008 F03254 F00581 alpha 667794 BA36097 AA293803 gamma 671008 F03254 F00581 alpha 671909 AA421586 beta 671909 AA421586 beta 671909 AA421586 F01363 alpha 671909 AA421586 F01363 alpha 671909 AA421586 F01363 alpha 671909 F03254 F00581 alpha 671909 AA421586 F01363 alpha 671909 AA421586 F01363 alpha 671909 AA421586 F01363 alpha 671909 AA421586 F01363 alpha 671909 F03254 F00681 alpha 671909 F032				
171906				
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210768		1147502		
213616         H70324         H70325         alpha           236027         H62070         alpha           248153         N53829         N73325         alpha           24991         (T80528)         R39000         alpha           26298         R13508         (R20629)         alpha           265817         N28661         N21457         alpha           268628         N22757         alpha           27342         R37173         alpha           290344         (N50305)         N47094         alpha           290894         N72005         alpha           290894         N72005         alpha           307767         W21278         alpha           340635         W56712         W56757         alpha           340683         W55986         W56276         alpha           346647         W94390         W74638         alpha           359349         AA010546         AA010547         alpha           359349         AA010546         AA010547         alpha           39915         R50455         beta           40764         R56327         R56245         alpha           49811				
238027         H62070         alpha           248153         N53829         N73325         alpha           24991         (T80528)         R39000         alpha           26298         R13508         (R20629)         alpha           265817         N28661         N21457         alpha           266828         N22757         alpha           27342         R37173         alpha           280344         (N50305)         N47094         alpha           290894         N72005         alpha           290894         N72005         alpha           307787         W21278         alpha           340635         W56772         alpha           340635         W56772         alpha           340683         W55986         W56276         alpha           346647         W94390         W74638         alpha           346796         W79685         W79784         alpha           359349         AA010546         AA010547         alpha           364632         AA022809         AA022690         alpha           364632         AA022809         AA02545         alpha           45086         H08908				
248153         N53829         N7325         alpha           24991         (T80528)         R39000         alpha           26298         R13508         (R20629)         alpha           265817         N28661         N21457         alpha           268628         N22757         alpha           27342         R37173         alpha           280344         (N50305)         N47094         alpha           290894         N72005         alpha           294142         N68597         alpha           307787         W21278         alpha           340635         W56712         W56757         alpha           340635         W56712         W56757         alpha           340635         W56712         W56757         alpha           340635         W55986         W56276         alpha           346647         W94390         W74638         alpha           346796         W79685         W79784         alpha           359349         AA010546         AA010547         alpha           364832         AA022809         AA022699         alpha           364832         AA022809         AA022699         alpha			H7U325	
24991         (T80528)         R39000         alpha           26298         R13508         (R20629)         alpha           265817         N28661         N21457         alpha           266828         N22757         alpha           27342         R37173         alpha           280344         (N50305)         N47094         alpha           290894         N72005         alpha           294142         N68597         alpha           307787         W21278         alpha           340635         W56712         W56757         alpha           340633         W55986         W56276         alpha           346647         W94390         W74638         alpha           346796         W79685         W79784         alpha           359349         AA010546         AA010547         alpha           359349         AA022809         AA022690         alpha           39915         R56327         R56245         alpha           49632         R56327         R56245         alpha           45866         H08824         alpha           45806         H08824         alpha           45020			NITOOOF	
26288         R13508         (R20629)         alpha           265817         N28661         N21457         alpha           266828         N22757         alpha           27342         R37173         alpha           280344         (N50305)         N47094         alpha           290894         N72005         alpha           290894         N72005         alpha           307787         W21278         alpha           340635         W56712         W56757         alpha           340635         W56712         W56757         alpha           340635         W55986         W56276         alpha           346647         W94390         W74638         alpha           346796         W79685         W79784         alpha           359349         AA010546         AA010547         alpha           359349         AA010546         AA010547         alpha           364632         AA022809         AA022690         alpha           49764         R56327         R56245         alpha           49811         H2906         H28976         alpha           49807         H10267         H10213         alpha <td></td> <td></td> <td></td> <td></td>				
265817         N28661         N21457         alpha           286828         N22757         alpha           27342         R37173         alpha           280344         (N50305)         N47094         alpha           290894         N72005         alpha           294142         N68597         alpha           307767         W21278         alpha           340635         W56712         W56757         alpha           340683         W55986         W56276         alpha           346647         W94390         W74638         alpha           346796         W79685         W79784         alpha           359349         AA010546         AA010547         alpha           359349         AA012809         AA022690         alpha           359349         AA012560         AA022690         alpha           359349         AA010546         AA010547         alpha           359349         AA022809         AA022690         alpha           39915         R50455         beta           40764         R56327         R56245         alpha           45086         H08908         H08824         alpha      <				
268628         N22757         alpha           27342         R37173         alpha           280344         (N50305)         N47094         alpha           290894         N72005         alpha           294142         N68597         alpha           307767         W21278         alpha           340685         W56712         W56757         alpha           340683         W55986         W56276         alpha           346647         W94390         W74638         alpha           346647         W94390         W74638         alpha           346796         W79685         W79784         alpha           359349         AA010546         AA010547         alpha           364632         AA022809         AA022690         alpha           39915         R50455         beta           40764         R56327         R56245         alpha           49807         H10267         H10213         alpha           49811         H29080         H28976         alpha           49811         H29080         H28976         alpha           66473         R16018         R16119         alpha <td< td=""><td></td><td></td><td></td><td></td></td<>				
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290894         N72005         alpha           294142         N68597         alpha           307767         W21278         alpha           340635         W56712         W56757         alpha           340683         W55986         W56276         alpha           346647         W94390         W74638         alpha           346796         W79585         W79784         alpha           359349         AA010546         AA010547         alpha           364632         AA022809         AA022690         alpha           39915         R50455         beta           40764         R56327         R56245         alpha           45086         H08908         H08824         alpha           49811         H29080         H28976         alpha           50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         alpha           69907         T48654         T48655         alpha           739014         (AA42185)         AA421567         beta           739014         (AA42185)         AA421567         beta				
294142         N68597         alpha           307787         W21278         alpha           340635         W56712         W56757         alpha           340683         W55986         W56276         alpha           346647         W94390         W74638         alpha           346796         W79585         W79784         alpha           359349         AA010546         AA010547         alpha           359349         AA022809         AA022690         alpha           39915         R50455         beta           40764         R56327         R56245         alpha           45086         H08908         H08824         alpha           48607         H10287         H10213         alpha           49811         H29080         H28976         alpha           50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         alpha           69907         T48654         T48655         alpha           739009         AA421586         beta           739014         (AA42185)         AA421567         beta	280344	(N50305)		
307787   W21278   alpha			N72005	alpha
340635         W56712         W56757         alpha           340683         W55986         W56276         alpha           346647         W94390         W74638         alpha           346796         W79585         W79784         alpha           359349         AA010546         AA010547         alpha           364632         AA022809         AA022690         alpha           39915         R50455         beta           40764         R56327         R56245         alpha           45086         H08908         H08824         alpha           48607         H10267         H10213         alpha           49811         H29080         H28976         alpha           50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         alpha           687794         AA258686         AA258608         alpha           69907         T48654         T48655         alpha           72391         AA394097         AA293803         parrma           739009         AA421586         beta           771303         AA421567         alpha	294142		N68597	
340683         W55986         W56276         alpha           346647         W94390         W74638         alpha           346796         W79585         W79784         alpha           359349         AA010546         AA010547         alpha           364632         AA022809         AA022690         alpha           39915         R50455         beta           40764         R56327         R56245         alpha           45086         H08908         H08824         alpha           48807         H10287         H10213         alpha           49811         H29080         H28976         alpha           50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         alpha           687794         AA258686         AA258608         alpha           69907         T48654         T48655         alpha           72391         AA394097         AA293803         garmma           739014         (AA42185)         AA421567         beta           771303         AA421567         alpha           2-5         L36675         alpha	307787	W21278		
346647         W94390         W74638         alpha           346796         W79585         W79784         alpha           359349         AA010546         AA010547         alpha           364632         AA022809         AA022690         alpha           39915         R50455         beta           40764         R56327         R56245         alpha           45086         H08908         H08824         alpha           48607         H10267         H10213         alpha           49811         H29080         H28976         alpha           50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         alpha           667794         AA258686         AA258608         alpha           69907         T48654         T48655         alpha           73901         AA394097         AA293803         gamma           739014         (AA42185)         AA421567         beta           771303         AA443638         gamma           2-4         L36675         alpha           c-1rb08         F03254         F06981         alpha	340635	W56712	W56757	alpha
346796         W79585         W79784         alpha           359349         AA010546         AA010547         alpha           364632         AA022809         AA022690         alpha           39915         R50455         beta           40764         R56327         R56245         alpha           45086         H08908         H08824         alpha           48607         H10267         H10213         alpha           49811         H29080         H28976         alpha           50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         alpha           667794         AA258686         AA258608         alpha           69907         T48654         T48655         alpha           73909         AA421586         beta           739014         (AA42185)         AA421567         beta           771303         AA443638         garmma           2-4         L36675         alpha           c-1rb08         F03254         F06981         alpha           c-2td12         F08836         F11169         alpha	340683	W55986	W56276	· · · · · · · · · · · · · · · · · · ·
359349         AA010546         AA010547         alpha           364632         AA022809         AA022690         alpha           39915         R50455         beta           40764         R56327         R56245         alpha           45086         H08908         H08824         alpha           48607         H10267         H10213         alpha           49811         H29080         H28976         alpha           50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         alpha           687794         AA258686         AA258608         alpha           69907         T48654         T48655         alpha           73901         AA394097         AA293803         garmma           739014         (AA42185)         AA421567         beta           771303         AA443638         garmma           2-4         L36675         alpha           c-1rb08         F03254         F06981         alpha           c-2td12         F08836         F11169         alpha           c-28f08         F03751         F07521         alpha <td>346647</td> <td>W94390</td> <td>W74638</td> <td>alpha</td>	346647	W94390	W74638	alpha
364632         AA022809         AA022690         aipha           39915         R50455         beta           40764         R56327         R56245         aipha           45086         H08908         H08824         aipha           48607         H10267         H10213         aipha           49811         H29080         H28976         aipha           50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         aipha           687794         AA258686         AA258608         aipha           69907         T48654         T48655         aipha           72391         AA394097         AA293803         garmna           739009         AA421586         beta           739014         (AA42185)         AA421567         beta           771303         AA443838         garmna           2-4         L36675         alpha           c-1rb08         F03254         F06981         alpha           c-2td12         F08836         F11169         alpha           c-28f08         F03751         F07521         alpha	346796	W79585		alpha
39915         R50455         beta           40764         R56327         R56245         alpha           45086         H08908         H08824         alpha           48607         H10267         H10213         alpha           49811         H29080         H28976         alpha           50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         alpha           687794         AA258686         AA258608         alpha           69907         T48654         T48655         alpha           72391         AA394097         AA293803         gamma           739009         AA421586         beta           771303         AA421567         beta           771303         AA443638         gamma           2-4         L36675         alpha           c-01f06         F01363         alpha           c-1rb08         F03254         F06981         alpha           c-2td12         F08836         F11169         alpha           c-26f08         F03751         F07521         alpha           cDNA         S69965         bet	359349	AA010546	AA010547	alpha
40764 R56327 R56245 alpha 45086 H08908 H08824 alpha 48607 H10267 H10213 alpha 49811 H29080 H28976 alpha 50202 H17962 beta 50470 H16811 beta 66473 R16018 R16119 alpha 687794 AA258686 AA258608 alpha 69907 T48654 T48655 alpha 72391 AA394097 AA293803 gamma 739009 AA421586 beta 739014 (AA42185) AA421567 beta 771303 AA443638 gamma 2-4 L36675 alpha 2-5 L36674 alpha c-01f06 F01363 alpha c-1rb08 F03254 F06981 alpha c-2td12 F08836 F11169 alpha c-2t618 F03751 F07521 alpha cDNA S69965 gamma EST01420 M78265 gamma EST01420 M78265 gamma	364632	AA022809	AA022690	alpha
45086         H08908         H08824         alpha           48607         H10267         H10213         alpha           49811         H29080         H28976         alpha           50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         alpha           687794         AA258686         AA258608         alpha           69907         T48654         T48655         alpha           72391         AA394097         AA293803         gamma           739009         AA421586         beta           739014         (AA42185)         AA421567         beta           771303         AA443838         gamma           2-4         L36675         alpha           2-5         L36674         alpha           c-01f06         F01363         alpha           c-1rb08         F03254         F06981         alpha           c-2td12         F08836         F11169         alpha           c-28f08         F03751         F07521         alpha           cDNA         S69965         beta           EST01420         M78265 <td< td=""><td>39915</td><td></td><td>R50455</td><td>beta</td></td<>	39915		R50455	beta
48607         H10267         H10213         alpha           49811         H29080         H28976         alpha           50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         alpha           687794         AA258686         AA258608         alpha           69907         T48654         T48655         alpha           72391         AA394097         AA293803         gamma           739009         AA421586         beta           739014         (AA42185)         AA421567         beta           771303         AA443838         gamma           2-4         L36675         alpha           2-5         L36674         alpha           c-01f06         F01363         alpha           c-1rb08         F03254         F06981         alpha           c-2td12         F06836         F11169         alpha           c-28f08         F03751         F07521         alpha           cDNA         S69965         beta         gamma           EST01420         M78265         gamma           (HRBAA27)         beta <t< td=""><td>40764</td><td>R56327</td><td>R56245</td><td>alpha</td></t<>	40764	R56327	R56245	alpha
49811         H29080         H28976         alpha           50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         alpha           687794         AA258686         AA258608         alpha           69907         T48654         T48655         alpha           72391         AA394097         AA293803         gamma           739009         AA421586         beta           739014         (AA42185)         AA421567         beta           771303         AA443638         gamma           2-4         L36675         alpha           2-5         L36874         alpha           c-01f06         F03254         F06981         alpha           c-1rb08         F03254         F06981         alpha           c-2td12         F08836         F11169         alpha           c-28f08         F03751         F07521         alpha           cDNA         S69965         beta           EST01420         M78265         gamma           (HRBAA27)         EST19193         AA317129         beta	45086	H08908	H08824	aipha
50202         H17962         beta           50470         H16811         beta           66473         R16018         R16119         alpha           687794         AA258686         AA258608         alpha           69907         T48654         T48655         alpha           72391         AA394097         AA293803         gamma           739009         AA421586         beta           739014         (AA42185)         AA421567         beta           771303         AA443838         gamma           2-4         L36675         alpha           2-5         L36874         alpha           c-01f06         F03254         F06981         alpha           c-1rb08         F03254         F06981         alpha           c-2td12         F08836         F11169         alpha           c-28f08         F03751         F07521         alpha           cDNA         S69965         beta           EST01420         M78265         gamma           (HRBAA27)         EST19193         AA317129         beta	48607	H10267	H10213	alpha
50470         H16811         beta           66473         R16018         R16119         alpha           687794         AA258686         AA258608         alpha           69907         T48654         T48655         alpha           72391         AA394097         AA293803         gamma           739009         AA421586         beta           739014         (AA42185)         AA421567         beta           771303         AA443638         gamma           2-4         L36675         alpha           2-5         L36874         alpha           c-01f06         F01363         alpha           c-1rb08         F03254         F06981         alpha           c-2td12         F08836         F11169         alpha           c-28f08         F03751         F07521         alpha           cDNA         S69965         beta           EST01420         M78265         gamma           (HRBAA27)         EST19193         AA317129         beta	49811	H29080	H28976	alpha
66473 R16018 R16119 alpha 687794 AA258686 AA258608 alpha 69907 T48654 T48655 alpha 72391 AA394097 AA293803 gamma 739009 AA421586 beta 739014 (AA42185) AA421567 beta 771303 AA443638 gamma 2-4 L36675 alpha 2-5 L36874 alpha c-01f06 F01363 alpha c-1rb08 F03254 F06981 alpha c-2td12 F08836 F11169 alpha c-28f08 F03751 F07521 alpha cDNA S69965 beta EST01420 M78265 gamma beta	50202		H17962	beta
687794 AA258686 AA258608 alpha 69907 T48654 T48655 alpha 72391 AA384097 AA293803 gamma 739009 AA421586 beta 739014 (AA42185) AA421567 beta 771303 AA443638 gamma 2-4 L36675 alpha 2-5 L36874 alpha c-01f06 F01363 alpha c-1rb08 F03254 F06981 alpha c-2td12 F08836 F11169 alpha c-2ef08 F03751 F07521 alpha cDNA S69965 beta EST01420 M78265 (HRBAA27) EST19193 AA317129 beta	50470		H16811	beta
69907         T48654         T48655         alpha           72391         AA394097         AA293803         gamma           739009         AA421586         beta           739014         (AA42185)         AA421567         beta           771303         AA443638         gamma           2-4         L36675         alpha           2-5         L36674         alpha           c-01f06         F01363         alpha           c-1rb08         F03254         F06981         alpha           c-2td12         F08836         F11169         alpha           c-26f08         F03751         F07521         alpha           cDNA         S69965         beta           EST01420         M78265         gamma           (HRBAA27)         beta	66473	R16018	R16119	
72391 AA384097 AA293803 gamma 739009 AA421586 beta 739014 (AA42185) AA421567 beta 771303 AA443638 gamma 2-4 L36675 slipha 2-5 L36674 slipha c-01f06 F01363 slipha c-1rb08 F03254 F06981 slipha c-2td12 F08836 F11169 slipha c-2ef08 F03751 F07521 slipha cDNA S69965 beta EST01420 M78265 gamma (HRBAA27) EST19193 AA317129 beta	687794	AA258686	AA258608	alpha
739009 AA421586 beta 739014 (AA42185) AA421567 beta 771303 AA443638 gamma 2-4 L36675 slpha 2-5 L36674 slpha c-01f06 F01363 slpha c-1rb08 F03254 F06981 slpha c-2td12 F08836 F11169 slpha c-2ef08 F03751 F07521 slpha cDNA S69965 beta EST01420 M78265 gamma (HRBAA27) EST19193 AA317129 beta	69907	T48654	T48655	alpha
739014 (AA42185) AA421567 beta 771303 AA443638 gamma 2-4 L36675 elpha 2-5 L36674 elpha c-01f06 F01363 elpha c-1rb08 F03254 F06981 elpha c-2td12 F08836 F11169 elpha c-2ef08 F03751 F07521 elpha cDNA S69965 beta EST01420 M78265 gamma (HRBAA27) EST19193 AA317129 beta	72391	AA394097	AA293803	gamma
771303 AA443638 gamma 2-4 L36675 alpha 2-5 L36874 alpha c-01f06 F01363 alpha c-1rb08 F03254 F06981 alpha c-2td12 F08836 F11169 alpha c-28f08 F03751 F07521 alpha cDNA S69965 beta EST01420 M78265 gamma (HRBAA27) EST19193 AA317129 beta	739009	AA421586		beta
2-4 L36675 alpha 2-5 L36874 alpha c-01f06 F01363 alpha c-1rb08 F03254 F06981 alpha c-2td12 F08836 F11169 alpha c-28f08 F03751 F07521 alpha cDNA S69965 beta EST01420 M78265 gamma (HRBAA27) EST19193 AA317129 beta	739014	(AA42185)	AA421567	beta
2-5 L36674 alpha c-01f06 F01363 atpha c-1rb08 F03254 F06981 alpha c-2td12 F06836 F11169 alpha c-28f08 F03751 F07521 alpha cDNA S69965 beta EST01420 M78265 gamma (HRBAA27) EST19193 AA317129 beta	771303		AA443638	gamma
c-01f06         F01363         atpha           c-1rb08         F03254         F06981         alpha           c-2td12         F08836         F11169         alpha           c-28f08         F03751         F07521         alpha           cDNA         S69965         beta           EST01420         M78265         garmma           (HRBAA27)         beta	2-4		L36675	elpha
c-1rb08         F03254         F06981         alpha           c-2td12         F08836         F11169         alpha           c-28f08         F03751         F07521         alpha           cDNA         S69965         beta           EST01420         M79265         gamma           (HRBAA27)         beta	2-5		L36674	alpha
c-1rb08         F03254         F06981         alpha           c-2td12         F08836         F11169         alpha           c-28f08         F03751         F07521         alpha           cDNA         S69965         beta           EST01420         M79265         gamma           (HRBAA27)         beta			F01363	alpha
c-28f08         F03751         F07521         alpha           cDNA         S69965         beta           EST01420         M79265         garmma           (HRBAA27)         beta	c-1rb08	F03254	F06981	alpha
CDNA S69965 beta EST01420 M79265 gamma (HRBAA27) EST19193 AA317129 beta	c-2td12	F08836	F11169	alpha
CDNA S69965 beta EST01420 M79265 gamma (HRBAA27) EST19193 AA317129 beta		F03751	F07521	alpha
EST01420 M79265 gamma (HRBAA27) EST19193 AA317129 beta		S69965		beta
(HRBAA27)	EST01420	M79265		gamma
L3119193				
F0700040 AA040774	EST19193	AA317129		beta
ES122040   AA3197/4     Bipha	EST22040	AA319774		elpha

Figure 7 cont.

EST26845	T28079		beta
EST31489	AA328063		aipha
EST68G11	W22518		gamma
F1-625D	R29481		alpha
GEN-129D09	D61090		beta
hbc590	T11070		alpha
HIBBA65	T08213	T08212	aipha
	HR70E3R	HR70E3F	alpha
HSNACP0		U46896-	alpha
		46901	<u> </u>
KK1311	N83633		alpha
		D318839	alpha
		L08850	alpha
	T28735		alpha
	Z20502		alpha

Figure 8

10	20	30	40	50	60	70
CEGCCGCAGCCGCCG AGGGGCCCGGGAXAA TCCGCGGCCCTGGAG CAGAAGGGCXCCGCG GGGGCCAGTGCACCG	TDADDADDXAAA. DDDTDADDTTTTT	OTTODODD: DDDDTDAD: ADDDDDDDD	DOBCETOUL PROPERTIES TXTROCTOR	GGAAAGCGG GCGGGGAGGG	AGGGALGCTAG BAGGGGGCTCA BCTGGGGTGAG	AGTGC 210 AGTGC 280 AGGGCC 350
360	370	380	390	400	410	420
TGTCCATGGCCAAGC GAAGACCAAGGAGGC TCCCCCTACAGTGTC AGATGGGGCXAGGTC AATGGGGACACGGGC	GAGGGCGTTGTGG GCGTCCTCTACG GGAGCTGGGGCC CAXCAXGGGTCA GCGGGCTGATGA 720	CAGCEGEG TEGGTGGGE GGGTCCCGG TAAGGGACA GGTGGGGGG	GAGAAAACCA XGGGGGCXGG GGAGGGGGGT TACCCAXCCG CTCCAXCTGA 740	AGCAGGGGG GTTTCTGGG TCTGGGCAA ATAGAAXCC AAGGCCAGG 750	TCACCGAGGCG GCTGCAGGGCT GATAATATXAX TGGGTCTGTAT GACCAXTGCAX 760	GCGGA 420 GGGGG 490 TCAGC 560 CCGGA 630 TXATA 700 770
AAAXCACACAXCCTG GTATGCCAAGTACTG TAAGGGAAACTGGG AGCCAATTTCTTAG CCCAGGGCTGGGA	GGGTAAAATGTC CTTCCCATTGGT TGGTGGAACCAA AAAGTGAAGTG	ATAACATC( AGXTAAAT ACTGAGTT(	CATTTCCTCA TTTAGGTTCA CCATCCGTGA	IGTAATGCTT GAAAGGCTTG AACGGGGACA	AATTGAATGTC	ACAGG 840 AGTTC 910 CGCTT 980
1060	1070 			بينابيين	لتتتلبين	
ACATCCACTAGAGG GGCCCAGTGCAGTG CAGTGACGGGGCCA AGTCCTTAAATGTG CITCAGGTACTAGG	ATAAGACGGGGGGGGGGGGGGGGGGCCCCCCCCCCCCCC	TTAACATG TGTAATCA CCCLGCAG	GGGGTGCAGG CCATGCTGTG GAAGCAAGAE	TIGTAGGATX CTGGAGTTTC CCGAGAAGGT	CADDADDDDT CADTOOTTDT CAADATDDTDT	GCGCAG 1260 GTGTGG 1330
1410	1420	1	1	1		14.70
AGGGGGGGGGGGGGGGGGGGGGGGGGGGGAAAAAACCAGGACACAGGACAAAAAA	CATCGGTGTTTCC CAGGAACAGGCC CCCCCAACAGGC	CCCTGGCT CACATCTG ACGAATTC	CCCAAACUCC GGAGGAGCTC	TGTTCTCAACG TGTTCTCTGI TGAAGGTAAGG	GGGCAGGGAAC. CGATCCTTCTG ACCTGTCACCA	ATCGCA 1610 ACCCGC 1680 TCCCCG 1750
1760	1770	1780	1790 	1800 مىيلىيىل	1810 	1820
CCCCCCTATECT TCAGCTCAGAATG GCTGTCTGCGTGT TTGTTCATTCATT CCCTTTCAGCCXA	GCCACCAGCTTG CATCTGAATAAX ATCCTGCTTGCC	GAACACAA( GGCGTGCAT AGCGTGAC	GCCACTTTGCO GGGGTGTGACO JCATATGACT JCATGCCAXA TTGGGGTCCC 2140	CTCCCATCCT GCTCCCGGTG TCTGGCCACG XCCAGCCCTG GATGCCCAGC	TCTGCATGTGT TCCTTGAGCTT ACAGAGCCTGA 2160	CAATGA 1960 CCAGXT 2030 CACAAA 2100 2170
GGATGAGGCATAA CGGCGTGGAGAGG CCTCCATCCCACT CCCTAGCCTTCCC CTCACGAGTCCTC	GCTGGTGAXTGA CAGCTCCCAAT TCCAAGGCACTC CACTCCAACCCA GACCTTTTCTGCC 2470	CCAAATAAA CCCCAAAGC CCACAGCCA 2480	ATGGTGGAAG CCACTTCAAC TAACTGAATT TTACCACTGT GAGGAAGTGG	TGTGGAGGXT TGTGATTCGG AGAAATTATC GGGAATTTGG CCCAGGAAGG	CCTTGTTTTGCC GGGGGCATCCTC CTGCTGAAGAAC 2510	GGGGAG 2170 CCTTCA 2240 CAACCCA 2310 GGCTGTC 2380 CCACTGA 2450 2520
TTGAGCCCCTGA' GCCAGAGGCGTA' CCAGAGCCAGGG CCTCGGAGCCTG CAGGGCCAGGG	TGGAGCCAGAAGI GGGGCCCAGGAG. CTGTCCTTAGAC TGTTAGTGTCTG TGCGGTCGCGGC	TCCATCTG TGGGAGAGT TCCTTCTG TCCATCTG TGGGAGCC	ATGAGACCO NOACOACACCO NOATCATOTOTOTOTOTOTOTOTOTOTOTOTOTOTOTOTOT	ACCECAGGA ATTCTGTCC AGATCTTCCT CCGCCCGCGT AGTGTTGCCT 2850	TCCGCTCTGAGG TCCGCTCTGAGG TCCGCTCTGAGG TCAACCCCGGG TCCCATCCA	CCGCCCC 2590 GCAACCC 2660 GCATGGA 2730 GCGTCTG 2800
2810 	سيلسب	11111111		<u></u>	<u></u>	<u> </u>

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Figure 9

10	20	30	40	
AGGGAGATCCAGCTC TGCACACCCACCATG TCGCCAAGGAGGGXG GCAGGGGGTGACGGA GTCATGTATGTGGGA	GATGTETTUA TGGTGGGTGC ACCAGCTGAG	AGAGGGCTT GGTGGAAAAG AAGACCAAGG TTTTTAAAGA	SACCAA 120 SAGGGG 160 SAAGAA 200	
210	220	230 	240	
TAAATTAATTGTGAT	TAAAGTTG 2	223		

Figure 10

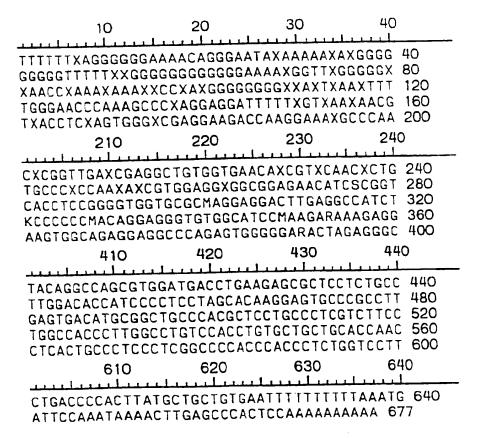


Figure 11

alpha-SYN exons 1-2

10	20	30	40
AATTTCAGCGATGCGA GTGTGAGCCACCTCCC CTCCCCAAGGGATAGC AGGCCCTCGNTCTCCC GGGTGGTCCCCNGGAC	AGGGCAAAGC CGGCGCTGCC CCTCTGCCCT CAGGNCGACT GGACCCAGAG	GCTCTCGGCG TGTCTCCTCC TGGTGGTCGA CTGACGAGGG GGAAAGGCNGG	GTGCG 40 AGCAG 80 CCCTC 120 GGTAGG 160 GGACAA 200 240
GAAGGAGGGAAGG AGCCCAACCGCTCCC CTAAACTTAACGTGA CCGCCTTGNNCCAGG CCCGCGCCCCCTGCC	GGAAAGAGGA GATCTCCACA GGCGCAAAA CAGGCGGCTC CCATCCCCA 420	AAGAGGCATCA AAGAGTGCTCO AAGAGTGCTCO AAGAGTTGATGO AAGAGATAGO 430	ATCCCT 240 STGACC 280 STTTTC 320 SCTCAC 360 GGACGA 400 440
GGAGCACGCTGCAGG GCGGGCAGAAGCGCT GCCGAGGAGAAGGAG ACGGCGACGACCAGA ACCGAGCGCCGCGAC	GAAAGCAGC GACAAATCA AAGGAGGAG AGGGGCCCA GCGAAGTGA	GAGCGCCGGG/ GCGGTGGGGG GACTAGGAGG/ AGAGAGGGGG	AGAGGG 440 CGGAGA 480 AGGAGG 520 CGAGCG 560 GGCTCA 600
GCGCAGACCCCGGCC CGCTCCCTCACGCCT CCCTCGTGAGCGGAG GGTTAGCGGGTTTGC CCGGCTCACAGCGGC	CGGCCCCTC TGCCTTCAA SAACTGGGAG CCTCCCACTC CCTCCTCTGG 820	CTGAGAGCGT GCCTTCTGCC TGGCCATTCG CCCCAGCCTC GGACAGTCCC 830	CCTGGG 640 TTTCCA 680 ACGACA 720 GCGTCG 760 CCCCGG 800 840
GTGCCCCTCCGCCC TTTCCTATTAAATA TTTTAAAAAAAAGAG GAGAAGCAGAGGGA CGGGNGTCTTTGGA	TTCCTGTGCG TTATTTGGGA AGAGGCGNGG CTCAGGTAAG AATCCTGGAG	CTCCTTTTCC AATTGTTTAAA AAGGAGTCGGA CACCTGTGGA AACGCCGGAT AACGCCGAT	TTCTTC 840 TTTTTTT 880 GTTGTG 920 TCTAAA 960 GGAGAC 1000
GAATGGTCGTGGGN GGACCGCTGGGCCA TTTGGGGAGCCTAA CCTGCTTCTGATAT TAGGCTGCTTCTCC	ACCGGGAGG( GGTCTCTGG( GGAAAGAGA( TCCCTTCTC	GGGTGGTGCTG GAGGTGAGTAC CTTGACCTGGC CACAAGGGCTC	GCCATGA 1040 CTTGTCC 1080 CTTTCGT 1120

Figure 11 cont.

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10	2	0	30	40
<del></del>		حسلسيا	سيلسيك	<del></del>
CTTAAAAGAGT	CTCACACT	TTGGAGGGTT	FTCTCATGAT	TT 40
TTCAGTGTTTT	TTGTTTAT	TTTTCCCCGA	AAAGTTCTCA	C8 TT
CAAAGTGTATT	TTATGTTT	TCCAGTGTGC	GTGTAAAGAA	AT 120
TCATTAGCCAT	GGATGTAT	TCATGAAAG	GACTTTCAAA	GG 160
CCAAGGAGGGA	GTTGTGGC	TGCTGCTGA	GAAAACCAAA	CA 200
210	2:	20 :	230	240
	<del></del>	<del>لىنىلىنى</del>	سينسين	
GGGTGTGGCAC	BAAGCAGCA	GGAAAGACA	AAAGAGGGTG	TT 240
CTCTATGTAGG	STAGGTAAA	CCCCAAATG	TCAGTTTGGT	GC 280
TTGTTCATGAC	STGATGGGT	TAGGATAAC	AATACTCTAA	AT 320
GCTGGTAGTT	CTCTCTCTT	GATTCATTT	TTGCATCATT	GC 360
TTGTCAAAAA	GGTGGACTG	AGTCAGAGG	TATGTGTAGG	TA 400
410	) 4	20	430	440
<del></del>	<del>.,,,</del>	<u> بيدليد با</u>	<u>ىيانىيان</u>	
GGTGAATGTG	AACGTGTGT	ATNTGAGCT.	AATAGTAAAA	AT 440
GCGACTGTTT	GCTTTTCAG	TAATTTTAAT	TTTGCCTAAT	AT 480
NTATGACTIN	TTAAAATGA	ATGTTTCTG	TACTACATAA	TT 520
CTATNTCAGA	GACAGT 53	6		

Figure 11 cont.

	10	20	30	40
ببيلييد	سيلسب		<u></u>	
CTGCAGGTC	CAACGGATCT	GTCTCTAGT	GCTGTACTTT	TAA 40
AGCTTCTAC	CAGTTCTGAA	ATTCAAAATT	ATCTTCTCAC	TGG 80
GCCCCGGTG	STTATCTCAT	TCTTTTTC	TCCTCTGTAA	GTT 120
			AAAGTCATTA	
TGTGCTAAA	ATCGTAATT	GGAGAGGAC	CTCCTGTTAG	CTG 200
_	210	220	230	240
<del></del>	بيليبيك		<del> </del>	
			AGGAGTTCCT	
TCTAGTTTT	AGGATATAT	ATATATATT	TTTTTCTTTC	CCT 280
GAAGATATA	TATATATA	ATACTTCTG	AGATTGAGA	TTT 320
TTAAATTAG	TTGTATTGA	AAACTAGCTA	AATCAGCAAT	TTA 360
AGGCTAGCT	TGAGACTTA	TGTCTTGAA	TTGTTTTTG	TAG 400
	110	420	430	440
OCTOOL	<del></del>	<del></del>	<del>uluutu</del>	
GUILLAAAA	CCAAGGAGG	GAGTGGTGCA	TGGTGTGGCA	AC 440
AGGIAAGCI	CCATTGTGC	TTATATCAA	GATGATATNI	ΓΑΑ 480
AGIATETAG	STGATTAGTG	TGGCCCAGTA	TCAAGATTC	CTA 520
TGAAATTGT	TAAAACAATC	ACTGAGCATO	TAAGAACATA	ATC 560
AGTCTTATT	GAAACTGAA	TTCTTTATA	AGTATTTT	AAA 600
6	310	620	630	640
	سيليسيل	<u> </u>	ورايرياب	ناب
TAGGTAAAT	ATTGATTAT	AAATAAAA	TATACTTGC	.VV 840
GAATAATGA	G 650		······································	777 040

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Figure 11 cont.

10		30	
ATATCTTAGCCAA ACTTGACATCTTG GTTATCTATGGCA TGGCTAGTGGAAG TACCACCCTTTAA	GATTCAATGTT GTGGCTTTIGT TGTGTAGATAC TGGAATGATTT	TGGTTGAACCA TTCTTCTGACC AGGTGTATGGA TAAGTCACTGT	CACTC 40 ACTCA 80 ANCGA 120 TATTC 160
	220	230	240
TGGCTGAGAAGAC AGCAGTGGTGACG GTGGAGGGAGCAG TCAAAAAGGACCA TTGTGTTACATTI	CAAAGAGCAAG GGTGTGACAGC GGAGCATTGCA GTTGGGCAAGG ATAAGCTGGTG 420	TGACAAATGTT AGTAGCCCAGA GCAGCCACTGG TATGGCTGTG AGATTACGGT 430	TGGAGG 240 AAGACA 280 GCTTTG 320 TACGTT 360 TCATTT 400 440
TCATGTGAAGCCT GGGAACGCTACC CCTTTATATTGG	TGGAGGCAGGAG CTGACCCTCCC	CAAGATACTT. CTTGTGAAAAA	ACTGTG 440

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Figure 11 cont.

10	20	30	40
AAAAGTTTACATACT CAATGTTTCCCCGGA TAGTAATATTAAGGT ACATCCCTATATGTA TTTTTAAAAAGTGAAA	TTGAGGTTGA GGCATTGTGG GTGCCATTTT AGATTTTTCC AATGCTACTT 220	TAACCCATGTI AGTTTAGAATO CAAGATCCGTO AAAACATGGT	GCCA 120 FCTGA 160
GTGCTTCTTACTTTA ACAGGAAGGAATTCT AATGAGGCTTATGAA CTGAATCTTTCTAAC GTCACATTTCTCTTT	AATATTAGAA GGAAGATATE ATGCCTTCTG AAGACAGTAG	TGAAGAAGGA CCTGTGGATC GAGGTAGGAGT CCAAAAACCTG	CTGAC 280 CCAAG 320 TCATT 360
GCTCTCTACATGCTC GAATAGTTTTTACAT AGGAGGAGGAAGATC GAAATCATATGTAGT TTGACCCTTTACAGC	ATTACGTGGA TTTTTAAAGGG GAAGAAGAGGA CCCACATAGC	GTCCTTAAAAA AAGAAAGGATG TTAATATACNT	TAAAA 520 ACTAC 560
GAGAATATATTTTT AGTGTAAAGTGGGG CAGTGCTGATGCGT GCTGTCT 727	AGCCATTTCC	TATCTCATTG	SCTGTC 680

10	20	30	40
TTTTGATTTTTCTAA AACCTGAAGCCTAAG AGATCTGCTGACAGA	TATTAGGAAG AAATATCTTT TGTTCCATCO	GGTATCAAGA GCTCCCAGTT TGTACAAGTG	TCTTG 80 SCTCAG 120
TTCCAATGTGCCCAG AGTGTATCTCGAAGT			
210	220	230	240
CTGTACCTGCCCCCA ACTGAAGTGAATACA GGATTTTGTGGCTTC AAACACCTAAGTGAC ATTTTTTTGTTGCTG	CTCAGCATTT TGGTAGCAGO AATCTACGAT TACCACTTAT	TCGGTGCTTCC GGTCTTTGTGT TGTTAAAACAA TTTCTAAATCC AGTTGTTAGTC	CCTTTC 240 IGCTGT 280 AATTAA 320 CTCACT 360 GATTIG 400
410	420	430 LL	440
CTATCATATATTATN ACTGTCTAAGAATAA TATATNATACTTAAA CTATAATACTAAATA TTTTATTCACTTGTG	TGACGTATT( AATATGTGA( TGAAATTTT	GTGAAATTTGT GCATGAAACT/ ACCATTTTGC(	TTAATA 480 ATGCAC 520 GATGTG 560
610	620	630	640
AATAAAACGTTATCT CCCATCTCACTTTAA CATGAATTAAGAACT TATTAATAGCCATTT TAGAGAAAATGGAAC	TCATTGCAAA ATAATAAAAA TGACACAAAG TGAAGAAGGA	AATATTTTAT TCATGCTTATA GACAAAAATA GGAATTTTAGA	TTTTAT 640 AAGCAA 680 TAAAGT 720 AAGAGG 760
810	820	830	840
GAAGCAACACTGCCA TCCTTAAGTGGCTG GAAGACCCCAACTAA TTCAATCCTGTCAA TGTTGTTTGATGTG	AGAAGTGTGT TGATTAATTA CTATTGTAGA TGTTTGCTTT	TTTGGTATGC TTGAAAGTGG GTGGTCTATT ACGTATTTTG	ACTGGT 840 GGTGTT 880 TCTCCC 920 GGGAAC 960
1010	1020	1030	1040
TTAATTGAGCCTTT TCGAAATAATTTTT TGGTGTGAATGCTG GACCATGAATAAAA CTAAGCAGTGTAGA	TATTAACATA TAGTTAAAAT TACCTTTCTG AAAAAAAAA	TATTGTTATT CTATTTTGTC ACAATAAATA AGTGGGTTCC	TTTGTC 1040 TGATAT 1080 ATATNC 1120 CGGGAA 1160

Figure 11 cont.

1210	1220	1230	1240
ليستنابين	<del>لىبىلىنى</del>	<del></del>	<del>Lilia.</del>
GAGAGCCATAAGACA	CATTAGCACA	TATTAGCACA	ATTCAA 1240
GGCTCTGAGAGAATG	TGGTTAACTT	TGTTTAACT	CAGCAT 1280
TCCTCACTTTTTTT	TTTAATCATC	AGAAATTCT	CTCTCT 1320
CTCTCTCTTTTTCTC	TEGETETETT	TTTTTTTT.	TTTTT 1360
TTTTACAGGAAATGC	CTTTAAACAT	CGTTGGGAA	CTACCA 1400
1410	1420	1430	1440
1410			, , , <del>,</del> , , <del>, , , , , , , , , , , , ,</del>
	ليسلسيا		Lill.
لسلسلسسا	GAGNATCAAT	TCTCTAGGA	CTGGAT 1440
GAGTCACCTTAAAGG	GAGNATCAAT CCTCCTTTAAA	TCTCTAGGA!	CTGGAT 1440 AAATAT 1480
GAGTCACCTTAAAGG AAAAATTTCATGGGG	GGAGNATCAAT CCTCCTTTAAA GTTTTTCCNTA	TCTCTAGGA ATGTTGCCC GGGGGAAGG	CTGGAT 1440 AAATAT 1480 GTTIIT 1520

Inter onal Application No. PCT/US 98/13071

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 012N15/12 007K14/47

01201/68

C12N15/11 A01K67/027 207K16/18

A61K48/00

According to international Patent Classification (IEC) or to poth halishal practition and IEC

G01N33/68

### B. FIELDS SEARCHED

Minimum documentation searched is assisted tinely tem to to keep by classification symbols IPC 6 C12N C07K A61K C12Q G01N A01K

Cocumentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search inamelor data base and iwhere practical isearch terms used)

Category	Citation of document, with indication, where appropriate. Mithe relevant passages	Relevant to claim No	
X	SCHAPIRA A. H.: "Pathogenesis of Parkinson's disease." BAILLERES CLINICAL NEUROLOGY. vol. 6, no. 1, April 1997, pages 15-36,	1-23. 57-61,74	
Y	xP002083889 see page 17. paragraph 2 see abstract	24-56. 62-73	
Υ	US 5 494 794 A (WALLACE DOUGLAS C) 27 February 1996 see the whole document	24-56. 62-73	
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other means "P" document published prior to the international filing date but later than the priority date claimed					
Date of the actual completion of the international search	Date of mailing of the international search report				
10 November 1998	27/11/1998				
Name and mailing address of the ISA	Authorized officer				
European Patent Office, P.B. 5818 Patentiaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2940, Tx. 31 651 epo nl Fax: (+31-70) 340-3016	Mandl. B				

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT					
Category	Citation of document, with indication where appropriate of the relevant passages	Belevant to claim No			
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gctaatcogcaatttaaggctagcttgogacttatgtettgootttgtttllgtogaCCTCCAAAACCAAGGAGGGAGTGGTGCATRGTGTGAACAGgtoagctccattg City Ser Lys Thr Lys Gliu Gily Val Val His Gily Val (Thr) Thr

tgcttatatccoogatgatatntaaagtatclagtgattogtgtggcccogtatcoogattcctatgooattgtoaaccaatcactgogcatctoogaacatatc

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#### (57) Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder with a lifetime incidence of approximately 2 percent. It was recently reported that a PD susceptibility gene is located on the long arm of human chromosome four. The present invention reports the subsequent identification of a mutation in the alpha synuclein gene, which codes for a presynaptic protein thought to be involved in neuronal plasticity. The finding of a specific molecular alteration which is causative for PD will permit the detailed understanding of the pathophysiology of the disorder, which will lead to potential therapeutic interventions, as well as a means for diagnosing individuals having an increased risk of developing the disease.

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### CLONING OF A GENE MUTATION FOR PARKINSON'S DISEASE

#### BACKGROUND OF THE INVENTION

#### Field of the Invention

Parkinson's disease (PD) is a common neurodegenerative disorder with a lifetime incidence of approximately 2 percent. A pattern of familial aggregation has been 10 documented for the disorder, and it was recently reported that a PD susceptibility gene in a large Italian kindred is located on the long arm of human chromosome 4. We have identified a mutation in the alpha synuclein gene, which codes for a presynaptic protein thought to be involved in 15 neuronal plasticity, in the Italian kindred and in three unrelated families of Greek origin with autosomal dominant inheritance for the PD phenotype. This finding of a specific molecular alteration which is causative for PD will permit the detailed understanding of the pathophysiology of the 20 disorder. In addition, methods of screening nucleic acids for the presence of mutations in the synuclein gene to test for predisposition to Parkinson's Disease are now possible.

#### 25 2. Technology Background

Parkinson's disease (PD) was first described by James

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Parkinson in 1817 (1). The clinical manifestations of this neurodegenerative disorder include resting tremor, muscular rigidity, bradykinesia and postural instability. A relatively specific pathological feature accompanying the neuronal degeneration is the intracytoplasmic inclusion body, known as the Lewy body, which is found in many regions including the substantia nigra, locus ceruleus, nucleus basalis, hypothalamus, cerebral cortex, cranial nerve motor nuclei, and the central and peripheral divisions of the autonomic nervous system (1).

In many cases a heritable factor predisposes to the development of the clinical syndrome (2). We have recently shown that genetic markers on human chromosome 4q21-q23 segregate with the PD phenotype in a large family of Italian descent (3). The clinical picture of the PD phenotype in the Italian kindred has been well documented to be typical for PD, including Lewy bodies, with the exception of a relatively earlier age of onset of illness at  $46 \pm 13$  years. In this family the penetrance of the gene has been estimated to be 85%, suggesting that a single gene defect is sufficient to determine the PD phenotype.

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We now report the identification of a mutation in the alpha synuclein gene that is associated with Parkinson's disease. The mutation, an Alab3Thr substitution, was found to be linked to the PD phenotype in four independent PD

families and absent from 314 control chromosomes, providing strong genetic evidence that this mutation in the human alpha synuclein gene is causative for the PD phenotype in these families.

The Ala53Thr substitution is localized in a region of the protein whose secondary structure predicts an alpha helical formation, bounded by beta sheets. Substitution of the alanine with threonine is predicted to disrupt the alpha helix and extend the beta sheet structure. Beta pleated sheets are thought to be involved in the self aggregation of proteins which could lead to the formation of amyloid like structures (6).

This was already tested in the case of NAC35, the 35 amino acid peptide derived from alpha-synuclein that was first isolated from plaques found in patients with Alpheimer's disease (4). NAC35 was shown to self aggregate and form amyloid fibril which shared the 'amyloid' characteristics of insolubility in aqueous solutions and green birefringence under polarized light, subsequent to Congo red staining (6). NAC35 is located in the middle of the alpha synuclein molecule and extends from amino acid 61 to amino acid 95. Residue 53, which is found to be mutated in FD, is outside the NAC35 peptide found in amyloid plaques. However, the true size of the NAC peptide involved in the plaques is not known since the protease used to

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isolate the peptide from AD tissue cuts at lysine 60 of the alpha synuclein protein. It is therefore possible that amino acid 53 may be part of the NAC peptide found in plaques. In crosslinking experiments with beta amyloid (Abeta), it was demonstrated (6) that residues 1-56 and 57-97 specifically bind amyloid and that a synthetic peptide consisting of residues 32-57 performed similarly.

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Three members of the synuclein family have been characterized in the rat, with SYN1 exhibiting 95% homology with the human alpha-synuclein protein (7). SYN 1 of the rat is expressed in many regions of the brain, with high levels found in the olfactory bulb and tract, the hippocampus, dentate gyrus, habenula, amygdala and piriform cortex, and with intermediate levels in the granular layer of the cerebellum, substantia nigra, caudate-putamen, and dorsal raphe (7). This pattern of expression coincides with the distribution of the Lewy bodies found in brains of patients with Parkinson's disease. It is also interesting to note that decrease in olfactory sense often accompanies the syndromic features of Parkinson's disease, and in many cases it is proposed that hyposmia is a prodromic sign of the illness (8).

In the zebra-finch the homologue to alpha synuclein, synuclein, is thought to be involved in the process of song learning, suggesting a role for synuclein perhaps in memory

and learning (9). In contrast to humans, rats have a threonine at residue 53 of their homologues to the human alpha synuclein gene (Figure 4). Similarly, the zebra-finch synelfin carries a threonine at amino acid 53, whereas both Bos taurus and Torpedo californica do not (10). There are no reports that suggest the presence of Lewy bodies in the brains of the rat or the zebra finch or a phenotype resembling that of PD. Lack of any phenotype could be explained by a combination of factors, including the following: the relative short life span of rodents may prohibit the observation of a late enset disorder, interaction with other cellular components not present in the rat may be required for the phenotype, absence of a critical environmental trigger in the rodents, or finally a heterozygous status Ala/Thr may be necessary for the production of a phenotype.

that missense mutations can cause an adult onset neurodegenerative disorder. Of the 31 mutations described so far in the loci for presentlin 1 and 2, thirty were missense and one was a splice variant (11). Missense mutations in the prion protein have also been implicated in the amylcid production seen in Gerstmann-Straussler-Scheinker and Creutzfeld-Jakob diseases, both forms of spongiform encephalopathy (12). Studies in these neurodegenerative

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disorders have pointed to the importance of the physical chemical properties of mutant cellular proteins in initiating and propagating neuronal lesions leading to disease. Similar studies in the synuclein protein family may provide valuable insights into the eticlogy and pathogenesis of PD.

patients with early onset Alzheimer's disease, the mutation identified in the alpha synuclein gene is unlikely to account for the majority of sporadic and familial cases of PD. However, this mutation may account for a significant proportion of those families with a highly penetrant, early onset autosomal dominant PD phenotype.

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

# 3. Summary of the Invention

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As described herein, we have discovered that particular mutations in the alpha synuclein gene are associated with predisposition to Parkinson's disease. Accordingly, the present invention includes an isolated nucleic acid comprising a mutated synuclein gene. In particular, the isolated nucleic acid of the present invention contains at

least one mutation in the alpha synuclein gene at base pair position 209 of Genbank # L08850, which, in particular, is a change from guanine to adenine. However, since other mutations in the alpha synuclein gene may also lead to Parkinson's Disease (PD), other mutations are also included. In addition, it is conceivable that mutations in the related beta (46)(SEQ ID NO 11) and gamma (SEQ ID NOS 12 and 13) synuclein genes may also lead to PD. Thus, mutated homelogues of the alpha synuclein gene are also included in the present invention. Vectors comprising the isolated nucleic acid and host cells comprising such vectors are included as well.

Knowledge of particular genes that are associated with PD allows for the search for other specific PD mutations.

Accordingly, the present invention also includes a method of using a synuclein gene sequence to identify specific PD mutations. Such mutations may occur in an unrelated population or in a family that demonstrates passage of PD within the family tree.

Since knowledge of mutations associated with Parkinson's disease allows the development of genetic screens that test for an individual's chances of being predisposed to the disease, and such tests may be performed by hybridization analysis using oligonucleotides complementary to the sequence of interest or by PCR amplification using oligonucleotides that are complementary to sequences flanking the mutation, the present invention also includes oligonucleotides

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complementary to a portion of the synuclein gene, wherein said portion comprises or flanks a mutation associated with predisposition to Parkinson's Disease. In particular, the oligonucleotides of the present invention will have a sequence that is complementary to a sequence from the alpha synuclein gene that includes or flanks base pair position 209. And in particular, this mutation is a change from guanine to adenine at this position.

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Mutated synuclein gene will allow the production and isolation of the mutant protein in an appropriate host cell using techniques well known in the art. Alternatively, peptides may be chemically synthesized using techniques also well known in the art. Isolation of such a protein or peptides thereof will allow the study of the molecular mechanisms which lead to development of Parkinson's disease. Accordingly, the present invention also includes an isolated synuclein protein or peptide containing at least one mutation. In particular, this mutation is at a position corresponding to the fifty-third amino acid in the native alpha synuclein protein, and in particular, this mutation is an alanine to threonine substitution.

Peptides corresponding to portions or the entirety of a synuclein gene may be useful as drugs for inhibiting the self-aggregation of mutant proteins that is thought to lead to Parkinson's disease. Accordingly, the present invention includes a method of testing peptides and other compounds for

aggregation can be tested using a number of established methods, including Congo red staining, electron microscopy pictures of amyloid fibrils, and circular dichroism (CD) spectrophotometry. Using a peptide derived from the alpha synuclein protein that includes the mutant THR amino acid at position 53 alone or in combination with a normal peptide may allow testing for drugs that can inhibit the aggregation or dissolve an aggregate. This procedure can be used to rapidly identify agents that could be used in animal studies, clinical trials, or as diagnostic tools.

Possession of isolated synuclein proteins or peptides will also allow the isolation of specific antibodies using techniques well known in the art. Such antibodies may distinguish a mutant synuclein protein from its wildtype counterpart, and therefor could also be used in diagnostic screens. Alternatively, such antibodies may also be used to inhibit the self-aggregation of proteins during the progression of Parkinson's disease. Accordingly, the present invention also includes antibodies specific for a mutated synuclein protein or peptide. It should be understood that useful derivatives of such antibodies, such as Fv fragments and Fab fragments, are also included.

The above aspects of the present invention will allow methods of detecting subjects at increased risk for Parkinson's Disease. Such a method comprises obtaining a sample comprising nucleic acids from the subjects, and

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detecting in the nucleic acids the presence of a mutation which is associated with Parkinson's disease. In particular, the mutation detected by the method of the present invention is located on human chromosome four, preferably in the alpha synuclein gene. In particular, the mutation causes an amino acid substitution at position 53 of the alpha synuclein gene, which is, in particular, an alanine to threonine substitution.

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The detecting step of the method of the present invention may be accomplished several different ways as will be described in further depth below. All such methods are well known to those of ordinary skill in the art.

For instance, said detecting step may comprise combining a nucleotide probe which selectively hybridizes to a nucleic acid containing a mutation associated with a predisposition to Parkinson's disease, and detecting the presence of hybridization. Such a probe may be an oligonucleotide that is complementary to a portion of the synuclein gene, wherein said portion comprises the mutation. In particular, such an oligonucleotide is complementary to a mutated alpha synuclein gene having at least one mutation at base pair position 209. In particular, this mutation is a change from guanine to adenine.

The detecting step of the method of the present invention may also comprise amplifying a nucleic acid product comprising said mutation, and detecting the presence of said mutation in the amplified product using any nucleic acid

sequencing procedure known in the art. Alternatively, the detecting step may comprise selectively amplifying a nucleic acid product comprising said mutation, and detecting the presence of amplification using any appropriate method known in the art. Such methods include gel electrophoresis of amplified nucleic acids, and detection of radiolabeled amplified nucleic acids using autoradiographic film or any other detection method known in the art.

The amplifying step of the present invention may be performed using the polymerase chain reaction (PCR), reverse transcriptase PCR (RTPCR), or any other type of PCR reaction known in the art. Accordingly, such a step will comprise at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids. In particular, said amplifying step uses two oligonucleotides. And in particular, the two oligonucleotides have the sequences given in SEQ ID NOS 2 and 3.

Alternatively, the detecting step of the method of the present invention comprises detecting the presence or absence of a restriction endonuclease site as detected by enzymatic digest of a nucleic acid sample. Such a detecting means will be possible when a mutation associated with a predisposition to Parkinson's disease results in a sequence having a new restriction endonuclease cleavage site, or loss of a native restriction endonuclease site. In particular, the mutation associated with Parkinson's disease results in the formation of a non-native Tsp45I restriction endonuclease site.

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Alternatively, the detecting step of the present invention may be performed using a gene-specific primer and subsequent chain termination at the position of the mutation using DNA polymerase and labeled nucleotides or dideoxynucleotides. The presence of nucleic acids in which a dideoxynucleotide corresponding to the mutation of interest is incorporated at the appropriate position may be detected by any means known in the art, including detection of radiolabeled dideoxynucleotides using, for example, autoradiographic film, or detection of fluorescently-labeled dideoxynucleotides.

Since the methods and compounds of the present invention will be useful in diagnostic screening precedures aimed at identifying individuals having a predisposition for Parkinson's disease, the present invention also includes diagnostic kits which include the compounds of the present invention in a form that allows such compounds to be used quickly and easily for the designated purpose.

Finally, the inventors also contemplate that the isolated nucleic acid, oligonuclectides and antibodies of the present invention may eventually be used in methods directed at the correction or suppression of Parkinson's disease. For example, cliqonuclectides or expression vectors designed from the synuclein nucleic acid sequences of the present invention may one day be used in antisense therapy directed at inhibiting expression of the mutated synuclein protein in

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patients with Parkinson's disease, or in individuals having a predisposition for Parkinson's disease. Similarly, antibodies specific for the mutated synuclein protein may be useful in therapies directed at inhibiting the self-aggregation of mutated proteins or peptides in patients having Parkinson's disease. Knowledge of gene(s) associated with the development of Parkinson's disease may also allow the design of transgenic animals which express the mutant gene(s). Such animals may serve as a useful disease model, allowing one to test the effects of candidate therapies and therapeutic compositions in the treatment or inhibition of Parkinson's disease.

A detailed description of the present invention is now provided, and should not be considered as limiting on the present invention as described above.

## 4. Brief Description of the Drawings

Figure 1.

DNA sequence of the PCR product used for mutation detection (SEQ ID NO 1). Cligonucleotide primers are shown by arrows and the numerals 3 and 13 (SEQ ID NO 2 and 3). Intron sequence is shown in lower case and exon sequence in upper case. Amino acid translation of the exon is shown below the DNA sequence. The circled base represents the G209A change in the mutant allele. The resulting amino acid

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Ala53Thr change is represented by the circled amino acid. The newly created Tsp45 I site is indicated above the DNA sequence.

### 5 Figure 2.

Mutation analysis of the G209A change is shown in a subpedigree of the Italian kindred. Filled symbols represent affected individuals. Numerical identifiers, denote the individuals immediately above. Tsp45 I digestion of PCR products is shown at the bottom of the rigure, and fragment sizes are indicated on the right in base pairs.

### Figure 3.

Mutation analysis of the G209A change in ET PCR products (7). Lane 1: 100 bp ladder, lanes 2 and 3 normal control, lanes 4 and 5 PD patient, lane 6 negative control without RT enzyme. Sizes are indicated on the right in base pairs. Lanes 2 and 4 show uncut DNA and lanes 3 and 5 show DNA cut with Tsp45 I.

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#### Figure 4.

Sequence alignments of alpha synuclein homologues in different species. Accession numbers for the sequences used were as follows: Homo sapiens Swiss-Prot P37840 (SEQ ID NO 4), Rattus norvegicus Swiss-Prot P37377 (SEQ ID NO 5), Bos

taurus Swiss-Prot P33567 (SEQ ID NO 6), Serinus canaria genbank L33860 (SEQ ID NO 7), Torpedo californica Swiss-Prot P37379 (SEQ ID NO 8). Numbering on top of the alignments is according to the human sequence. Amino acid 53, which is the site of the Ala53Thr change, is circled.

Figure 5.

The pedigree of a large family with PD (3). The clinical and pathological features of some members of this kindred were previously reported.

Figure 6.

Multipoint LCD score analysis between chromosome 4q markers and the PD locus.

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Figure 7.

A table of human synuclein clones identified from various databases. Columns labeled 5' and 3' show the sequence acquisition numbers. Clones were identified by homology to protein or nucleic acid sequence. Human gamma clones were identified by homology to known mouse and rat gamma synuclein sequences.

Figure 8.

25 Sequence of BAC clone 139A20 for human beta synuclein.

BAC clone was isolated using primers to known database sequences described in Figure 7. The sequence shown includes all coding exon sequences and some non-coding intronic sequences. (SEQ ID NO:11)

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Figure 9.

Sequence from the 5' end of BAC clone 174F13 for human gamma synuclein. The BAC clone was isolated with primers from the database sequences described in Figure 7.(SEQ ID NO:12)

Figure 10.

Sequence from the 3' end of BAC clone 174P13 for human gamma synuclein. BAC clone was isolated as described in Figure 9. The entire human gamma synuclein gene has now been sequenced and has been deposited in GenBank: accession number AF044311.(SEQ ID NO: 13) Figure 11.

Sequence of exons 1-7 of the human alpha synuclein gene, plus some flanking intronic sequence for each exon. (SEQ ID NOs 14-19)

# 5. Detailed Description of the Invention

### Definitions

Unless defined otherwise, all technical and scientific

terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. For purposes of the present invention, the following terms are defined below.

This invention provides a method of diagnosing or predicting a predisposition to Parkinson's disease. The method comprises detecting in a sample from a subject the presence of a mutation, for example, in nucleotide position 209 of the human alpha synuclein gene. The presence of the mutation indicates the presence of or a predisposition to Parkinson's disease.

As used herein, the term "gene" primarily relates to a coding sequence, but can also include some or all of the surrounding or flanking regulatory regions or introns. The term "gene" specifically includes artificial or recombinant genes created from cDNA or genomic DNA, including recombinant genes based upon splice variants.

As used herein, the term "synuclein" gene or protein may refer to the alpha synuclein gene or any homologue thereof.

A "homologue" is understood to mean any related gene or protein that is at least 25% homologous to the alpha synuclein gene or protein or performs a related function.

Preferably, a synuclein gene or protein refers to alpha, beta or gamma synuclein, but most preferably refers to alpha

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synuclein.

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As used herein, an "isolated nucleic acid" is a ribonucleic acid, deoxyribonucleic acid, or nucleic acid analog comprising a polynucleotide sequence that has been isolated or separated from sequences that are immediately contiguous, i.e. on the 5' and 3' ends, in the naturally occurring genome of the organism from which it is derived. The term therefor includes, for example, a recombinant nucleic acid which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule independent from any other sequences.

An isolated nucleic acid of the present invention may be "operatively linked" to an expression control sequence or regulatory region. As used herein, "operatively linked" means that the components are joined in such a way that the expression, transcription or translation of the sequence is under the influence or control of the regulatory region.

As used herein, a "predisposition" to Parkinson's disease means an increased probability of developing Parkinson's disease during the subject's lifetime as compared to the average individual.

Pertaining to this probability, a LOD score is a measure of genetic linkage used herein, defined as the log: ratio of the probability that the data would have arisen if the loci are linked to the probability that the data could have arisen from unlinked loci. The conventional threshold for declaring

linkage is a LOD score of 3.0, that is, a 1000:1 ratio (which must be compared with the 50:1 probability that any random pair of loci will be unlinked).

As used herein, reference to "base pair position" or "amino acid position" when referring to an isolated nucleic acid, probe, protein or peptide always indicates the relative position in the native gene or protein.

A "probe" refers to a nucleic acid which has sufficient nucleotides surrounding the codons at the mutation positions to distinguish the nucleic acid from nucleic acids encoding non-related genes. The specific length of the nucleic acid is a matter of routine choice based on the desired function of the sequence. For example, if one is making probes to detect the mutation in base pair position 209, the length of the nucleic acid is preferably small, but must be long enough to prevent hybridization to undesired background sequences. However, if the desired hybridization is to a nucleic acid which has been amplified, background hybridization is less of a concern and a smaller probe can be used. In general, such a probe will be between 10 and 100 nucleatides, especially between 10 and 40 and preferably between 15 and 25 nuclectides in length. It is apparent to one of skill in the art that nucleotide substitutions, deletions, and additions may be incorporated into the polynucleotides of the invention. However, such nucleotide substitutions, deletions, and additions should not substantially disrupt the ability of the polynucleotide to hybridize under conditions that are

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sufficiently stringent to result in specific hybridization.

As used herein with respect to genes, "the term "normal" refers to a gene which encodes a normal protein. As used herein with respect to proteins, the term "normal" means a protein which performs its usual or normal physiological role and which is not associated with, or causative of, a pathogenic condition or state. Therefor, the term "normal" is generally synonomous with the phrase "wild type".

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For any given gene or corresponding protein, a multiplicity of normal allelic variants may exist, none of which is associated with the development of a pathogenic condition or disease state. Such normal allelic variants include, but are not limited to, variants in which one or more nucleotide substitutions do not result in a change in the encoded amino acid sequence.

As used herein, the term "mutation" generally refers to a mutation in a gene that is associated with a predisposition to Parkinson's disease. "Mutant" can specifically refer to a mutation at nucleotide position 209 of the synuclein gene, and is in particularly a G to A transition. However, other mutations in the synuclein gene or other genes which are associated with a predisposition to Parkinson's disease are also encompassed. Furthermore, the term "mutation" is not limited to transition mutations, but can also mean a deletion, insertion or transversion as well.

The term "mutant", as it applies to synuclein genes, is not intended to embrace sequence variants which, due to the

degeneracy of the genetic code, encode proteins identical to the normal sequences disclosed or otherwise enabled herein; nor is it intended to embrace sequence variants which, although they encode different proteins, encode proteins which are functionally equivalent to normal synuclein proteins. The term "mutant" means a protein which does not perform its usual or normal physiological role and which is associated with, or causative of, a pathogenic condition or state.

since a mutation can be a substitution, deletion or insertion, a mutated synuclein "protein" is understood to refer to the amino acid sequence resulting from any such mutation whether the resulting protein is shorter, longer or modified, i.e. due to an alteration in reading frame or generation of stop codon. In addition, "peptide" is understood to refer to a portion of the mutated protein that is preferably at least five base pairs long, and more preferably at least 10 base pairs long. This portion may be derived from the amino or carboxyl terminus, or it may be an internal portion of the full length protein. As such, a peptide may be chemically synthesized using any method known in the art, or may be made using a recombinant DNA technology and an appropriate purification scheme or isolated from the native protein using enzymatic digestion.

As used herein, the term "substantially pure" means a preparation which is at least 60% by weight the compound of interest. Preferably the preparation is at least 75%, more

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preferably 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate method, i.e. column chromotography, gel electrophoresis or HPLC analysis.

"Specific or selective hybridization" as used herein means the formation of hybrids between a probe nucleic acid (e.g., a nucleic acid which may include substitutions, deletions, and/or additions) and a specific target nucleic acid (e.g., a nucleic acid having the mutated sequence), wherein the probe preferentially hybridizes to the specific target such that, for example, a band corresponding to the mutated DNA or restriction fragment thereof can be identified on a Southern blot, whereas a corresponding normal or wild-type DNA is not identified or can be discriminated from a variant DNA on the basis of signal intensity. Hybridization probes capable of specific hybridization to detect a single-base mismatch may be designed according to methods known in the art (13-17).

"Stringent" as it refers to hybridization conditions is a term of art understood by those of ordinary skill to refer to those conditions of temperature, chaotrophic acids, buffer and ionic strength which permit hybridization of a particular nucleic acid sequence to its complementary sequence and not to substantially different sequences. The exact conditions which constitute "stringent" conditions depend on the nature of the nucleic acid sequence, the length of the sequence, and the frequency of occurrence of subsets of that sequence

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within other non-identical sequences. By varying hybridization conditions from a level of stringency at which non-specific hybridization occurs to a level at which only specific hybridization occurs, one of ordinary skill in the art can, without undue experimentation, determine conditions which will allow a given sequence to hybridize only with complementary sequences.

Suitable ranges of stringency conditions are described in Sambrook et al. (13). Hybridization conditions, depending on the length and commonality of a sequence, may include temperatures of 20°C-65°C and ionic strengths from 5X to 0.1X SSC. Highly stringent hybridization conditions may include temperatures as low as 40°C-42°C (when denaturants such as formamide are included) or up to 60°C-65°C in ionic strengths as low as 0.1X SSC. These ranges are, however, only illustrative and, depending on the nature of the target sequence, and possible future technological developments, may be more stringent than necessary. Appropriate conditions may be determined for each specific nucleic acid sequence or oligonucleotide probe using standard control and a level of experimentation that is not considered to be undue by those of skill in the art.

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As discussed below in greater detail, the mutation can be detected by many methods. For example, the detecting step

can comprise combining a nucleotide probe capable of selectively hybridizing to a nucleic acid containing the mutation with a nucleic acid in the sample and detecting the presence of hybridization. Additionally, the detecting step can comprise amplifying the nucleotides surrounding and including the mutation and detecting the presence of the mutation in the amplified product, or selectively amplifying the nucleotides of the mutation and detecting the presence of amplification. Finally, the detecting step can comprise detecting the presence or absence of a restriction fragment created by an enzyme digest of the sample nucleic acid, or any other detection means known in the art.

### Detection Techniques

Once the location of a PD-relevant mutation is known, the methods to detect such a mutation are standard in the art. The sequence of various nucleotide probes can be determined from the known sequence of the relevant gene, especially the sequences surrounding the mutation.

Detection of point mutations using direct probing involves the use of oligonucleotide probes which may be prepared, for example, synthetically or by nick translation. The probes may be suitably labeled using, for example, a radio label, enzyme label, fluorescent label, biotin-avidin label and the like for subsequent visualization by any appropriate assay, i.e. Southern blot hybridization. In this procedure, the labeled probe is reacted with sample DNA that is bound, for example,

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to a nylon filter under conditions such that only fully complementary sequences hybridize. The areas that carry DNA sequences complementary to the labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling may then be visualized, for example, by autoradiography.

Methods of manipulating hybridization conditions to achieve varying degrees of specificity are well known in the art. For example, tetra-alkyl ammonium salts may be used to bind selectively to A-T base pairs, thus displacing the dissociation equilibrium and raising the melting temperature. At 3M Me 4NCl, this is sufficient to shift the melting temperature to that of G-C pairs. This results in a marked sharpening of the melting profile. The stringency of hybridization in such an experiment is usually 5°C below the Ti (the irreversible melting temperature of the hybrid formed between the probe and its target sequence) for the given chain length. For a 20mer oligonucleotide probe, the recommended hybridization temperature is about 58°C. The washing temperatures are unique to the sequence under investigation and need to be optimized for each variant.

There are certainly other ways known in the art for adjusting hybridization conditions in view of desired specificity. For instance, although hybridization may be carried out in accordance with conventional hybridization methods under suitable conditions with respect to e.g. stringency, incubation time, temperature, etc, the choice of

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conditions will depend on the desired degree of complementarity between the fragments to be hybridized. A high degree of complementarity requires more stringent conditions such as low salt concentrations, low ionic strength of the buffer and higher temperatures, whereas a low degree of complementarity requires less stringent conditions, e.g. higher salt concentration, higher ionic strength of the buffer or lower temperatures, for the hybridization to take place.

The support to which DNA or RNA fragments of the sample to be analyzed are bound in denatured form is preferably a solid support and may have any convenient shape. Thus, it may, for instance, be in the form of a plate, e.g. a thin layer or a microtiter plate, a strip, a solid particle e.g. in the form of a bead such as a latex bead, a filter, a film or paper. The solid support may be composed of a polymer, preferably nylon or nitrocellulose.

Alternative probing techniques, such as ligase chain reaction (LCE), may involve the use of mismatch probes, i.e., probes which are fully complementary with the target except at the point of the mutation. The target sequence is then allowed to hybridize both with oligonucleotides which are fully complementary and have oligonucleotides containing a mismatch, under conditions which will distinguish between the two. By manipulating the reaction conditions according to the above considerations, it is possible to obtain hybridization only where there is full complementarity. If a mismatch is

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present there is significantly reduced hybridization.

The polymerase chain reaction (PCR) is a technique that amplifies specific DNA sequences with remarkable efficiency. Repeated cycles of denaturation, primer annealing and extension carried out with Taq polymerase, e.g., a heat stable DNA polymerase, leads to exponential increases in the concentration of desired DNA sequences. Given a knowledge of the nucleotide sequence of the mutations, synthetic oligonucleotides can be prepared which are complementary to sequences which flank the DNA of interest. Each oligonucleotide is complementary to one of the two strands. The DNA is denatured at high temperatures (e.g., 95°C) and then reannealed in the presence of a large molar excess of oligonuclectides. The oligonucleotides, oriented with their 3' ends pointing towards each other, hybridize to opposite strands of the target sequence and prime enzymatic extension along the nucleic acid template in the presence of the four deoxyribonucleotide triphosphates. The end product is then denatured again for another cycle. After this three-step cycle has been repeated several times, amplification of a DNA segment by more than one million-fold can be achieved. The resulting DNA may then be directly sequenced in order to locate any genetic alteration. Alternatively, it may be possible to prepare oligonuclectides that will only bind to altered DNA, so that PCR will only result in multiplication of the DNA if the mutation is present. Following PCR, direct visualization or allele-specific oligonucleotide

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hybridization (18) may be used to detect the Farkinson's disease point mutation. Alternatively, PCR may be followed by restriction endonuclease digestion with subsequent analysis of the resultant products.

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As shown in the examples, the substitution of G for A at base pair 209 of the synuclein, results in the gain of a Tsp45I site. The gain of this restriction endonuclease recognition site facilitates the detection of the Parkinson's disease mutation using restriction fragment length polymorphism (RFLP) analysis or by detection of the presence or absence of the restriction site in a PCR product that spans base pair position 209.

For RFLP analysis, DNA is obtained, for example from the blood cells of the subject suspected of having Parkinson's disease and from a normal subject, is digested with a restriction endonuclease, and subsequently separated on the basis of size by agarose gel electrophoresis. The Southern technique can then be used to detect, by hybridization with labeled probes, the products of endonuclease digestion. The patterns obtained from the Southern blot can then be compared. Using such an approach, an additional restriction endonuclease site, such as a Tsp45I site, is detected by determining the number of bands detected and comparing this number to the normal subject.

The creation of a new restriction site as a result of a nucleotide substitution at a disclosed mutation site can be

readily determined by reference to the genetic code and a list of nucleotide sequences recognized by restriction endonucleases (19).

In general, primers for PCR are usually about 20 bp in length, and are most preferably 15-25 bp. Denaturation of strands usually takes place at 94°C. and extension from the primers is usually at 72°C. The annealing temperature varies according to the sequence under investigation. Examples of reaction times are: 20 mins denaturing; 35 cycles of 2 min, 1 min, 1 min for annealing, extension and denaturation; and finally a 5 min extension step.

pcr "amplification of specific alleles" (PASA) may also be used to detect the presence of the PD mutation. PASA is a rapid method of detecting single-base mutations or polymorphisms (22-28). PASA (also known as allele specific amplification) involves amplification with two oligonucleotide primers such that one is allele-specific. The desired allele is efficiently amplified, while the other allele(s) is poorly amplified because it mismatches with a base at or near the 3' end of the allele-specific primer. Thus, PASA or the related method of PAMSA may be used to specifically amplify the mutation sequences of the invention. Where such amplification is done on genetic material (or RNA) obtained from an individual, it can serve as a method of detecting the presence of the mutations.

As mentioned above, a method known as ligase chain reaction (LCR) can be used to successfully detect a

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single-base substitution (29, 30). LCR probes may be combined or multiplexed for simultaneously screening for multiple different mutations. Thus, LCR can be particularly useful where multiple mutations are predictive of the same disease.

Finally, the Parkinson's disease mutation of the present invention may also be detected using chain termination with labeled dideoxynucleotides. For instance, U.S. Patent No. 5,047,519 to Hobbs et al. discloses fluorescently-labeled nuclectides as chain-terminating substrates for a fluorescence-based DNA sequencing method. With such substrates and knowledge of the gene sequence of interest, it is possible to design an assay using a gene-specific primer to initiate a polymerase reaction immediately flanking the position of the mutation, employing color-coded dideoxynucleotide terminators such that the specific nucleotide at the position of the mutation may be easily determined via a colorimetric assay.

## 20 Transgenic Animals and Cell Lines

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Having identified subjects having a predisposition to Parkinson's disease associated with a specific mutation, the subjects can participate in the screening of putative agents capable of treating Parkinson's disease. This method comprises administering the test agent to the subject, which may be a human, which has a mutation in a gene associated with Parkinson's disease and monitoring the effect of the

agent on the subject's condition. If the symptoms of Parkinson's disease improve, the agent can be used as a treatment for the disease.

In addition, it is possible to develop transgenic model systems and/or cell lines containing the mutated nucleic acid(s) for use, for example, as model systems for screening for drugs and evaluating drug efficiency. Additionally, such model systems provide a tool for defining the underlying biochemistry of, for instance, the mutated synuclein gene, thereby providing a rationale for drug design.

One approach to creating transgenic animals is to mutate the animal gene of interest by in vivo mutagenesis, transfer the mutant gene into embryonic stem cells by DNA transfection and inject the embryonic stem cells into blastocysts in order to retrieve offspring which carry the disease-causing mutation (31). Alternatively, the technique of microinjection of the mutated gene, into a one-cell embryo followed by incubation in a foster mother can be used. Alternatively, viral vectors, e.g., Adeno-associated virus, can be used to deliver the mutated gene to a stem cell, or may be used to target specific cells of a fully developed animal (32,33).

# Antibodies and Recombinant Expression of Polypeptides

When the mutated gene product is a polypeptide, e.g. the 209 mutation, it can be used to prepare antisera and monoclonal antibodies using, for example, the method of Kohler and Milstein (34). Such monoclonal antibodies could

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then form the basis of a diagnostic test, or may even be useful in therapies directed toward inhibiting the action of the mutant protein in a patient with Parkinson's disease.

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Mutant polypeptides can also be used to immunize an animal for the production of polyclonal antiserum (35). For example, a recombinantly produced fragment of a variant polypeptide can re injected into a mouse along with an adjuvant so as to generate an immune response. Murine immunoglobulins which specifically bind the recombinant fragment can be harvested from the immunized mouse as an antiserum, and may be further purified by affinity chromatography or other means. Additionally, spleen cells are harvested from the mouse and fused to myeloma cells to produce a bank of antibody-secreting hybridoma cells, which can then be screened for clones that secrete immunoglobulins which bind the recombinantly produced fragment with an increased affinity. More specifically, immunoglobulins that selectively bind to the variant polypeptides but poorly or not at all to wild-type polypeptides are selected, either by pre-absorption with wild-type proteins or by screening of hybridoma cell lines for specific idiotypes that bind the variant but not wild-type.

These antibodies can be used to screen protein and tissue samples for the presence of mutated proteins. A colored enzymatic reaction occurs when the specific antibody remains bound to its target protein, in situ, after thorough washing, as directed by established protocols.

### Gene expression

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The nucleic acid sequences of the present invention will be capable of expressing the desired mutant or normal polypeptides in an appropriate host cell. For expression in host cells, the DNA sequences of the present invention will be operably linked to, i.e., positioned to ensure the functioning of, an expression control sequence. For example, such polynucleotides can include a promoter, a transcription termination site (polyadenylation site in eukaryotic expression hosts), a ribosome binding site, and, optionally, an enhancer for use in eukaryotic expression hosts. In addition, the DNA sequence of the present invention may also be fused such that the reading frame is conserved to an appropriate signal sequence to facilitate expert of the encoded protein across the cell membrane.

Expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. A variety of suitable expression vectors are disclosed in Sambrock et al. (13). Commonly, expression vectors will contain selection markers, e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells transformed with the desired DNA sequences.

E. coli is one prokaryotic host that is particularly useful for cloning and expression of the DNA sequences of the present invention because of the wide variety of available expression systems. Vectors suitable for use in E. coli are

known and are commercially available, i.e. pBR322 (13), pBLUESCRIPT (Stratagene), etc. Also, a variety of different types of expression systems may be used, including plasmids, cosmids, bacteriophage lambda, etc. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilus, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. Expression vectors for use in prokaryotic host cells will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any of a variety of well-known promoters may be used, such as the lactose promoter system, a tryptophan (Trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. A promoter may optionally contain an operator sequence for regulatable gene expression, and will have a ribosome binding site sequence for the initiation of translation.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (36). Vectors for use in sukaryotic cells are known and commercially available, i.e. pcDNA3 (Invitrogen). Eukaryotic cells are actually preferred, and a number of suitable host cell lines capable of secreting intact human proteins have been developed in the art, including CHO cells, COS cells, HeLa cells, myeloma cell lines, Jurkat cells, etc. Promoters for use in sukaryotic vectors may be cell-specific, or capable of being expressed

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in a wide variety of cells, i.e. viral promoters.

Expression vectors of the present invention (e.g., comprising nucleic acid sequences encoding a mutant or normal polypeptide) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts.

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#### Kits

The method lends itself readily to the formulation of test kits which can be utilized in diagnosis. Such a kit would comprise a carrier compartmentalized to receive in close confinement one or more containers wherein a first container may contain suitably labeled DNA probes. Other containers may contain reagents useful in the localization of the labeled probes, such as enzyme substrates. Still other containers may contain restriction enzymes (such as Tsp45I), buffers, etc., together with instructions for use.

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## DESCRIPTION OF THE INVENTION

# Detailed Description of the Preferred Embodiments

The following laboratory procedures were used:

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DNA samples were collected upon informed consent. High molecular weight genomic DNA was isolated from whole-blood

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lysate by methods previously described (38). Genotyping was performed as previously described (39). Pairwise linkage analysis was performed using the MLINK program of the FASTLINK package (40-42). Allele frequencies were used as reported in the Genomic Data Base (http://gdbwww.gdb.org) and the Cooperative Human Linkage Consortium (CHLC) database (http://www.chlc.org). Multipoint analysis was performed using the LINKMAP program of the FASTLINK package. For the multipoint analysis allele frequencies were set to 1/n where n equals the number of alleles observed. In the two point analysis LOD scores were calculated for both the reported and the 1/n allele frequencies with minimal effect on the maximum LOD score observed. Simulations of multipoint analysis in a subset of the pedigree with different allele frequencies similarly indicated no significant effect on the scores calculated. Maximum LOD scores as shown were observed for the heterozygote and homozygote disease allele penetrance set to 0.99, which is similar to the PD allele penetrance previously reported ranging from 0.88 to 0.94 (3). All unaffected individuals used in the study were of age above the mean for onset of illness. Disease allele frequency was set to 0.0001. Distances and order of genetic markers were set as reported in the CHLC database. Overlapping three point analysis was performed for markers D4S2361, D4S1647,  ${\it D4S421}$  and the PD locus. The 12 allele  ${\it D4S2380}$  locus was not included because of prohibitive time run. Multipoint

analysis was performed on an IBM SP2 parallel computer and the SGI Challenge machine.

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For mutation analysis genomic DNA was amplified with oligonucleotides (3): 5' GCTAATCAGCAATTTAAGGCTAG 3' (SEQ ID NO 3) of MO 2) and (13): 5' GATATGTTCTTAGATGCTCAG 3' (SEQ ID NO 3) of genbank ID: U46898, under standard PCR conditions. Sequence analysis was performed using the Perkin Elmer dye terminator cycle sequencing kit on an ABI 373 fluorescent sequencer (ABI, Foster City, CA). Restriction digestion was performed following the PCR with Tsp45 I according to manufacturer's protocol (New England Biolabs, Beverly, MA). The digested PCR products were electrophoresed on a 6% Visigel (Stratagene, La Jolla, CA), and visualized by ethidium bromide staining. Pedigree structure in Figure 2 has been slightly modified in order to protect patient confidentiality.

Total RNA was extracted from the lymphoblastoid cell line of an affected individual and first strand synthesis was performed by oligo dT priming (Gibco BRL, Gaithersburg, MD).

Primers (1F) 5' ACGACAGTGTGGTGTAAAGG 3' (SEQ ID NO 9) and (13R) 5' AACATCTGTCAGCAGATCTC 3' (SEQ ID NO 10) corresponding to nucleotides 21-40 and 520-501 of genbank L08850 were used to amplify a product of 500 bp containing the mutation at nucleotide 209. PCR products were subjected to restriction digestion by Tsp45 I. The mutation at nt 209 creates a novel Tsp45 I site (Figure 1), so that the normal allele will be

restricted in 4 fragments of 249, 218, 24 and 9 bp, where the mutant allele will have 5 fragments of 249, 185, 33, 24 and 9 bp of size, as shown in Figure 3. Size standards used, were the 100 bp ladder (Gibco BRL, Gaithersburg, MD).

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### Example 1

In an effort to identify a genetic locus responsible for Parkinson's disease, we performed a genome scan in a large kindred of Italian descent with pathologically confirmed PD (Figure 5). The kindred originated in the town of Contursi in the Salerno province of Southern Italy (3). Some members emigrated to the United States, Germany and other countries. The extended family pedigree consists of 592 members with 60 individuals affected by PD. The average age of onset for the illness in this pedigree (Figure 5) has been shown to be 46 ± 13 years. One hundred and fourty genetic markers were typed in this pedigree at an average spacing of about 20 cm. Genetic markers at the cytogenetic location 4q21-q23 were the only ones to show linkage to the disease phenotype with a Zmax=6.00 at theta=0.00 for marker D4S2380I (see Table 1).

Table 1. Two point LOD scores between chromosome 4q markers and the PD locus

	Two-point LOD scores at recombination fractions of:								0
Locus		0.01	0.05	0.10	0.20	0.30	0.40	$Z_{max}$	$\theta_{\text{max}}$
D4S2361	-5.60	-0.83	0.30	0.54	0.43	0.21	0.06	0.55	0.12
D4S2380	6.00	5.90	5.30	4.60	3.00	1.50	0.5(	ธ์.00	0.00
D4S1647		5.07	4.47	3.71	2.26	1.05	0.30	5.22	0.00
D4S421	-2.42	0.45	0.77	0.65	0.38	0.22	0.09	0.77	0.05

Recombinations between the disease phenotype and genetic markers were observed in the proximal region for marker D4S2361 and in the distal region for marker D4S421. Genetic markers D4S2380 and D4S1647 showed no obligate recombination events in the affected individuals.

Multipoint LOD score analysis between markers D4S2361-13cM-D4S1647-3cM-D4S421 and the disease locus places the PD gene between markers D4S2361 and D4S421 at a recombination distance of 0.00 cM from marker D4S1647 with a Zmax=6.04 (Figure 6). This location is favored from the alternative genetic intervals by a difference in the LOD score of greater than three LOD units.

Although expansions of unstable trinucleotide repeats are found in a number of human neurogenerative conditions, there is no evidence for an association of a CAG trinucleotide repeat expansion in families with PD (43). In

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addition, genetic linkage studies in other families with PD-like illnesses do not support the involvement of several candidate genes (glutathione peroxidase, tyrosine hydroxylase, brain-derived neurotrophic factor, catalase, amyloid precursor protein, CuZn superoxide dismutase and debrisequinone 4-hydroxylase) in the ethology of the disorder (44). Genes previously mapped in the general region of linkage include the loci for alcohol dehydrogenase, formaldehyde dehydrogenase, synuclein, UDP-N-acetylglycosamine phosphotransferase and others.

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Our localization of a PD susceptibility gene represents the first genetic locus linked to PD. Other distinct clinicopathological entities associated with parksonian features are probably linked to other genetic loci. For example, Wilhelmsen-Lynch disease (disinhibition-dementiaparkinsonian-amyotrophy complex) is linked to the 17q21-q22chromosomal region (45). If the pathogenesis of diseases affecting the nigrostriatal pathway includes environmental influences, then a range of mutations affecting vulnerable sites in the electron transport chain or enzyme polymorphisms influencing neurotoxin metabolism may vary the penetrance of PD by altering an individual's resistance to exogenous or endogenous agents. However, our finding of a highly penetrant genetic locus linked to PD suggested that abnormalities of a single gene may be sufficient to cause Parkinson's disease.

### Example 2

In an effort to identify a specific gene between markers D4S2361 and D4S421 that is associated with predisposition to Parkinson's disease, we conducted sequence analysis of candidate genes in this region.

Alpha synuclein, a presynaptic nerve terminal protein, was originally identified as the precursor protein for the NAC peptide, a non beta amyloid component of Alzheimer's disease (AD) amyloid plaques (4). The human alpha synuclein gene was previously mapped in the 4q21-q22 region (5). We refined the mapping, and determined that the alpha synuclein gene is located within the non-excluded region harboring the PD gene in the Italian kindred. Thus alpha synuclein represented an excellent candidate locus for PD.

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Sequence analysis of the fourth exon of the alpha synuclein gene revealed a single base pair G209A change from the published sequence of the gene (GenBank ID L0885C), which results in an Ala53Thr substitution and the creation of a novel Tsp45 I restriction site (Figure 1). Mutation analysis for the G209A change in the Italian kindred shows complete segregation with the FD phenotype with exception of individual 30 (Figure 2), who is affected but not carrying this mutation. This individual apparently inherited a different FD mutation from his father, as we have shown that

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he shares a genetic haplotype with his unaffected maternal uncle, individual 3, for genetic markers in the PD linkage region.

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The frequency of this variation was studied in two general population samples, one consisting of 120 chromosomes of the parents of the CEPH reference families, and the other consisting of 194 chromosomes of unrelated individuals from the blood bank in Salerno, Italy, a city near the town from which the family originated. Of these 314 general population chromosomes none was found to carry the G209A mutation. Fifty two patients of Italian descent with sporadic PD were also screened for the mutation (Figure 2), along with 5 propands from previously unpublished Greek families with PD. The Ala53Thr change was found to be present in three of the Greek kindreds and it segregated with the PD phenotype. In those three Greek kindreds it is worth noting that the age of onset for the disease is relatively early, ranging from the mid 30's to the mid 50's. Extended haplotype analysis of the Greek kindreds and the Italian PD family suggests that the mutations arose independently on different ancestral chromosomes. The finding of the Ala53Thr substitution in four independent PD families and its absence from 314 control chromosomes provides the strongest genetic evidence that this mutation in the human alpha synuclein gene is causative for

the FD phenotype in these families. 25

We have also demonstrated by RT PCR that the mutant allele is transcribed in the lymphoblast cell line of an affected individual from the Italian kindred (Figure 3) (7). Thus, it is reasonable to assume that the mutant protein is indeed expressed.

### Example 3.

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Since homologous genes that are related to the alpha synuclein protein have been identified in other species, it seemed reasonable to assume that homologues of alpha synuclein would exist in humans as well. In fact, human beta synuclein has previously been described (46), and is approximately 60% similar to alpha synuclein at the protein level.

We set out to identify other related homologues by searching various databases for homologous genes and proteins. Protein sequence databases searched included the NR (non-redundant) and "month" databases of Genbank and Swiss Prot. Nucleotide databases included NR, menth, dbstf, GSS (Genome Sequence Service) and EPD (eurkaryotic Promoter Database). Several human clones were identified and characterized as alpha, beta and gamma clones as shown in Figure 7. Potential gamma clones were identified on the basis of homology to known rat and mouse sequences. Although gamma synuclein has been identified in species other than

human, this is the first identification of the corresponding gamma synuclein from humans.

Using two primers sets designed from known database sequences (5'ATGTCTTCAAGAAGGGCTTC3'; 5'COTTGGTCTTCTCAGCTGCT3' and 5'AGCGTGGATGACCTGAAGAG3'; 5'AGCACAGGTGGACAGGCCAAG3',, we have isolated two BAC clones, 139A20 and 174P13, from a Genome System commercial Bacterial Artificial Chromosome library (St. Louis, MO: which contain the human beta and gamma synuclein genes, respectively. The beta gene contained one clone 139A20 has been sequenced as shown in Figure % (SEQ ID NO 11), which contains all coding exon sequences and some additional non-coding intronic sequence. The gamma clone 174P13 has been sequenced and is available in GenBank: accession number AF044311. Sequence from the 5' end is given in Figure 9 (SEQ ID NO 12), and sequence from the 3' end is given in Figure 10 (SEQ ID NO 13). The human alpha synuclein gene has also been sequenced as shown in Figure 11, which provides the sequence of each separate exon region with some additional flanking intronic sequence for each exon.

(SEQ ID NOs 14-19) 20

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The three numan homologues are highly conserved at the protein level. The alpha and beta human homologues have about 60.4% similarity. And the gamma homologue is about 38.3% and 32.8% similar to the alpha and beta homologues, respectively, based on the portion of the coding sequence

that we have obtained thus far. Thus, it is reasonable to presume that mutations in either the beta or gamma synuclein gene may also result in Parkinson's disease.

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  - 49. This application is based on provisional application number 60/505,684 filed June 25, 1997 which is relied upon and hereby expressly incorporated by reference herein.

## SEQUENCE LISTING

5	(1) GENERAL INFORMATION:
J	(i) APPLICANT: Polymeropoulus, Mihael Lavedan, Christian
	Leroy, Elisabeth
1.0	Nussbaum, Robert
10	Johnson, William Duvoisin, Roger
	buvoisin, kogei
	(ii) TITLE OF INVENTION: Cloning of a gene mutation for
3 -	Parkinson's disease
15	(iii) NUMBER OF CHOUPMORE. 30
	(iii) NUMBER OF SEQUENCES: 10
	(iv) CORRESPONDENCE ADDRESS:
	(A) ADDRESSEE: SPENCER & FRANK
20	(B) STREET: 1100 New York Ave. Suite 300 East
	(C) CITY: Washington
	(D) STATE: D.C.
	(E) COUNTRY: USA
25	(F) ZIP: 20005-3955
20	(v) COMPUTER READABLE FORM:
	(A) MEDIUM TYPE: Floppy disk
	(B) COMPUTER: IBM PC compatible
	(C) GPERATING SYSTEM: PC-DOS/MS-DOS
30	(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
	(vi) CURRENT APPLICATION DATA:
	(A) APPLICATION NUMBER: (B) FILING DATE: 25-JUN-1998
35	(C) CLASSIFICATION:
90	(C) CHASSIFICATION.
	(viii) ATTORNEY/AGENT INFORMATION:
	(A) NAME: Schneller, John W.
	(B) REGISTRATION NUMBER: 26,031
40	(C) REFERENCE/DOCKET NUMBER: NIH 0082A
	(ix) TELECOMMUNICATION INFORMATION:
	(A) TELEPHONE: (202)414-4000
	(B) TELEFAX: (202)414-4040
45	
	(a) Typenia Tray per and TR No 7
	(2) INFORMATION FOR SEQ ID NO:1:
	(i) SEQUENCE CHARACTERISTICS:
50	(A) LENGTH: 216 base pairs
	(B) TYPE: nucleic acid
	(C) STRANDEDNESS: double
	(D) TOPOLOGY: linear

DNCDCVCC .W/C corcores. .

	(ii) MOLECULE TYPE: DNA (genomic)	
	(iii) HYPOTHETICAL: NO	
5	(iv) ANTI-SENSE: NO	
10	<pre>(vii) IMMEDIATE SOURCE:     (B) CLONE: alpha synuclein gene/ exon 4 region</pre>	
10	(viii) POSITION IN GENOME:  (A) CHROMOSOME/SEGMENT: 4  (B) MAP POSITION: 4q21-q22	
15		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:	
6.0	GCTAATCAGC AATTTAAGGC TAGCTTGAGA CTTATGTCTT GAATTTGTTT TTGTAGGCTC	60
20	CAAAACCAAG GAGGGAGTGG TGCATGGTGT GACAACAGGT AAGCTCCATT GTGCTTATAT	120
	CAAAGATGAT ATNTAAAGTAT CTAGTGATTA GTGTGGCCCA GTATCAAGAT TCCTATGAA	181
25	ATTGTAAAACA ATCACTGAGC ATCTAAGAAC ATATC	216
	(2) INFORMATION FOR SEQ ID NO:2:	
30	(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
35	<pre>(ii) MOLECULE TYPE: other nucleic acid     (A) DESCRIPTION: /desc = "primer #3"</pre>	
	(iii) HYPOTHETICAL: NO	
40		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:	
45	GCTAATCAGC AATTTAGGCT AG	23
	(2) INFORMATION FOR SEQ ID NO:3:	
50	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 21 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	

(ii) MOLECULE TYPE: other nucleic acid (A) DESCRIPTION: desc = "primer #13" (iii) HYPOTHETICAL: NO (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3: 10 21 CTATACAAGA ATCTACGAGT C (2) INFORMATION FOR SEQ ID NO:4: (i) SEQUENCE CHARACTERISTICS: 15 (A) LENGTH: 140 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: not relevant (D) TOPOLOGY: not relevant 20 (ii) MOLECULE TYPE: peptide (iii) HYPOTHETICAL: NO (iv) ANTI-SENSE: NO 25 (vi) ORIGINAL SOURCE: (A) ORGANISM: Homo sapiens (C) INDIVIDUAL ISOLATE: Swiss-Prot P37840 30 (vii) IMMEDIATE SOURCE: (B) CLONE: alpha synuclein protein 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4: Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val 10 4.0 Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val 45 Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr 5.0 Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys 50 Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys

95 90 85 Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile 105 5 Leu Glu Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro 125 120 115 Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala 135 10 (2) INFORMATION FOR SEQ ID NO:5: (i) SEOUENCE CHARACTERISTICS: 15 (A) LENGTH: 140 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: not relevant (D) TOPOLOGY: not relevant (ii) MOLECULE TYPE: peptide 20 (iii) HYPOTHETICAL: NO (iv) ANTI-SENSE: NO 25 (vi) ORIGINAL SOURCE: (A) ORGANISM: Rattus norvegicus (C) INDIVIDUAL ISOLATE: Swiss-Prot P37377 (vii) IMMEDIATE SOURCE: 30 (B) CLONE: alpha synuclein protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5: 35 Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Gly Lys 40 20 25 Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val 45 Val His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr 55 Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys 50 Thr Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Phe Val Lys 9.0

	Lys A	.sp G		et G 00	ly L	ys G	ly G		lu G .05	ly T	yr F	ro G	eln o	31u (	Sly I	le
<b>(</b> )	Leu C		.sp N	let P	ro V	al A		ro S .20	er S	Ser C	Blu A	ala 1	Cyr ( 125	3lu M	Met P	ro
	Ser (	3lu 0 130	Glu G	sly T	Tyr G		.sp :	Tyr (	Blu F	Pro (		Ala 140				
10	(2) INFORM	ATIC	ON FO	R SI	EQ II	ON 0	6:									
15	(±) \$	(A) (B) (C)	LENC TYPE STRA	STH: E: ar ANDEI	RACTE 134 mino ONESS Y: no	amir acio S: no	io a: i ot r	cids elev	ant							
	(ii)	MOLE	CULE	TYP	E: pe	eptio	de									
20	(iii,	HYPO'	THET	ICAL	: NO											
	(iv)	ANTI	-SEN	SE:	NO											
25	(vi)	(A)	CRG.	ANIS	RCE: M: B UAL				ss-P	rot	P335	67				
30	(vii)		DIAT CLO		URCE alph		nucl	ein	prot	ein						
30	(V11)	(B)	CLO	NE :	alph	a sy										
35	(x1)	(B)	CLO	NE: DES	alph CRIP	a sy TION	: SE	EQ II	NO:	6:	Ala	Lys	Glu	Gly	Val 15	Val
35	(x1) Met 1	(B) SEQU	CLO ENCE Val	NE: DES	alph CRIP Met	a sy TION Lys	: SE Gly	IQ II Leu	) NO: Ser	6: Met 10					15	
	(x1) Met 1 Ala	(B) SEQU Asp	CLO JENCE Val Ala	NE: DES Phe Glu 20	alph CRIP Met 5 Lys	a sy TION Lys Thr	: SE Gly Lys	CQ II Leu Gln	Ser Gly 25	6: Met 10 Val	Thr	Glu	Ala	Ala 30	15 Glu	Lys
35	(x1) Met 1 Ala	(B) SEQU Asp Ala	CLO VENCE Val Ala Glu 35	DES Phe Glu 20	alph CRIP Met 5 Lys Val	a sy TION Lys Thr	: SE Gly Lys Tyr	CQ II Leu Gln Val 40	Ser Gly 25 Gly	6: Met 10 Val Ser	Thr Lys	Glu Thr	Ala Lys 45	Ala 30 Glu	Glu Gly	Lys Val
35 40	(x1) Met 1 Ala Thr	(B) SEQU Asp Ala Lys Gln	CLO VENCE Val Ala Glu 35	DES Phe Glu 20 Gly Val	alph CRIP Met 5 Lys Val	a sy TION Lys Thr Leu Ser	: SEGly Lys Tyr Val	Leu Gln Val 40 Ala	Ser Gly 25 Gly Glu	6: Met 10 Val Ser	Thr Lys	Glu Thr Lys	Ala Lys 45 Glu	Ala 30 Glu Gln	Glu Gly Ala	Lys Val Ser
35 40	(x1) Met 1 Ala Thr Val His 65	(B) SEQU Asp Ala Lys Gln 50	CLO VENCE Val Ala Glu 35 Gly	DES Fhe Glu 20 Gly Val	alph CRIP Met 5 Lys Val Ala	a sy TION Lys Thr Leu Ser Val 70	: SEGly Lys Tyr Val 55 Phe	Leu Gln Val 40 Ala Ser	Ser Gly 25 Gly Glu Gly	6: Met 10 Val Ser Lys	Thr Lys Thr Gly 75	Glu Thr Lys 60 Asn	Ala Lys 45 Glu Ile	Ala 30 Glu Gln Ala	Glu Gly Ala Ala	Lys Val Ser Ala

100 105 Glu Pro Glu Gly Glu Ser Tyr Glu Glu Gln Pro Gln Glu Glu Tyr Gln 120 5 Glu Tyr Glu Pro Glu Ala 130 (2) INFORMATION FOR SEQ ID NO:7: 10 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 142 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: not relevant 15 (D) TOPOLOGY: not relevant (ii) MOLECULE TYPE: peptide (iii) HYPOTHETICAL: NO 20 (iv) ANTI-SENSE: NO (vi) ORIGINAL SOURCE: (A) ORGANISM: Serinus canaria 25 (C) INDIVIDUAL ISOLATE: genbank L33860 (vii) IMMEDIATE SOURCE: (B) CLONE: alpha synuclein homologue 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7: Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Val Val Ala 35 Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys Thr 40 Lys Glu Gly Val Leu Tyr Val Gly Ser Arg Thr Lys Glu Gly Val Val 40 His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Ser Asn 50 45 Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys Thr 70 Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Leu Val Lys 50 85 Asp Gln Leu Ala Lys Gln Asn Glu Glu Gly Phe Leu Gln Glu Gly Met 100 105

	Val		Asn (	Thr (	Gly A	Ala A		Jal A 120	.sp I	Pro A	Asp A		Glu . 125	Ala :	Tyr C	slu
5	Met	Pro 130	Pro (	Glu (	Glu (		Fyr (	3ln /	Asp :	Tyr :		Pro 1	Glu .	Ala		
	(2) INFO	PMATI	ON F	OR S	EQ I	O <b>N</b> C	:8:									
10	(±)	(B)	ENCE LEN TYP STR TOP	GTH: E: a: ANDE	143 mino DNES	ami aci S: n	no a d ot r	cids elev	ant							
15	(ii)	MOLE	CULE	TYP	E: p	epti	de									
	(iii)	НТРО	THET	ICAL	: NO											
2.0	(±v)	ANT	-SEN	SE:	NO											
20	(vi)		GINAL ORG IND	ANIS	M: T	orpe					P373	79				
25	(vii)	IMM (B	EDIAT				muc]	ein	homo	ologi	ıe					
30	(xi)	SEQ'	UENCI	E DES	CRIE	10IT	1: SI	EQ II	NO:	:8:						
30		SEQ Asp									Ala	Lys	Glu	Gly	Val 15	Val
30	Me <sup>s</sup>		Val	Leu	Lys 5	Lys	Gly	Phe	Ser	Phe 10					15	
35	Me: 1	. Asp	Val Ala	Leu Glu 20	Lys 5 Lys	Lys Thr	Gly	Phe	Ser Gly 25	Phe 10 Val	Gln	Asp	Ala	Ala 30	15 Glu	Lys
	Me: 1 Al.	: Asp a Ala	Val Ala Gln 35	Leu Glu 20 Gly	Lys 5 Lys Val	Lys Thr Gln	Gly Lys Asp	Phe Gln Ala 40	Ser Gly 25 Ala	Phe 10 Val Glu	Gln Lys	Asp Thr	Ala Lys 45	Ala 30 Glu	Glu Gly	Lys Val
35	Med 1 Alo Th	: Asp Ala Lys	Val Ala Gln 35 Val	Leu Glu 20 Gly	Lys 5 Lys Val	Lys Thr Gln Lys	Gly Lys Asp Thr	Phe Gln Ala 40 Lys	Ser Gly 25 Ala Glu	Phe 10 Val Glu	Gln Lys Val	Asp Thr Val	Ala Lys 45 Gln	Ala 30 Glu Ser	Glu Gly Val	Lys Val Asn
35 40	Mei 1 Al. Th Me	Asp Ala Lys Tyr 50	Val Ala Gln 35 Val	Leu Glu 20 Gly Gly	Lys 5 Lys Val Thr	Lys Thr Gln Lys Thr 70	Gly Lys Asp Thr 55 Lys	Phe Gln Ala 40 Lys	Ser Gly 25 Ala Glu Gln	Phe 10 Val Glu Gly	Gln Lys Val Asn 75	Asp Thr Val 60 Val	Ala Lys 45 Gln Val	Ala 30 Glu Ser	Glu Gly Val Gly	Lys Val Asn Ala
35 40	Mei 1 Ale Th Me Th 65	Asp Ala Lys Tyr 50 r Val	Val Ala Gln 35 Val Thr	Leu Glu 20 Gly Gly Glu Gly	Lys 5 Lys Val Thr Lys Val 85 Ala	Lys Thr Gln Lys Thr 70 Asn	Gly Lys Asp Thr 55 Lys	Phe Gln Ala 40 Lys Glu Val	Ser Gly 25 Ala Glu Gln Ala	Phe 10 Val Glu Gly Ala Ser 90	Gln Lys Val Asn 75 Lys	Asp Thr Val 60 Val	Ala Lys 45 Gln Val	Ala 30 Glu Ser Gly	Glu Gly Val Gly Gly 95 His	Lys Val Asn Ala 80 Val

125 115 120 Glu Pro Leu Val Glu Ala Thr Glu Ala Thr Glu Glu Thr Gly Lys 130 135 5 (2) INFORMATION FOR SEQ ID NO:9: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 19 base pairs 10 (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cther nucleic acid (A) DESCRIPTION: /desc = "primer #1F" 15 (iii) HYPOTHETICAL: NO 20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9: 19 ACGACAGTGT GTGTAAAGG 25 (2) INFORMATION FOR SEQ ID NO:10: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs 30 (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: ther nucleic acid (A) DESCRIPTION: /desc = "primer #13R" 35 (iii) HYPOTHETICAL: NO 40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10: 20 AACATCTGTC AGCAGATETC 45 (2) INFORMATION FOR SEQ ID NO:11 (i) SEQUENCE CHARACTERISITCS (A) LENGTH: 2809 base pairs (B) TYPE: NUCLEIC ACID

- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAF.
- (ii) MOLECULAR TYPE: DNA (genomic)
- (iii) HYPOTHETICAL. NO
- 5 (iv) ANTI-SENSE: NO
  - (v) IMMEDIATE SOURCE:
    - (A) CLONE: BAC clone 139A20 HUMAN BETA SYNULEIN GENE
  - (vi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
- CCGCCGCAGC CGCCGCTCCA TCCCCAGCCC CGGCCCCGCA TCCGGTTTGG AAGGGGGCTG CAAGTTTGCA AGGGGCCCGG GANAAAAANC GAGCAGTGGC CCTTCCCGCG TCCCCAGGGT 10 TTCAAGGGAC GCTAGGANTN TCCGCGGCCC TGGAGGTTCG CACTGGGGAG TGGGGTGAGA TGGGGGJAAA GCGGGAGGG GCTCAGGGTC CAGAAGGGCN CCGCGGTCTC GGGAGTAGGG GGGCATNTGC GTCCCGCGGG AGGGGCTGGG GTGAGAGTGC GGGGCCAGTG CACCGGTGCC CGTGTATCGC CCTCCCCAGG CCGCCAGGAT GGACGTGTTC ATGAAGGGCC TGTCCATGGC CAAGGAGGGC GTTGTGGCAG CCGCGGAGAA AACCAAGCAG GGGGTCACCG AGGCGGCGGA 15 GAAGACCAAG GAGGGCGTCC TCTACGTCGG TGGGCNGGGG GCNGGGTTTC TGGGGCTGCA GGGCTGGGGG TCCCCCTACA GTGTGGAGCT GGGGCCGGGT CCCGGGGAGG GGGGTTCTGG GCAAGATAAT ATNANTCAGC AGATGGGGCN AGGTCANCAN GGGTCATAAG 3GACATACCC ANCCCATAGA ANCCTGGGTC TGTATCCGGA AATGGGGACA CGGGGCGGGC TGATGAGGTG GGGGGCTCCA NCTGAAAGGC CAGGGACCAN TGCANTNATA AAANCACACA NCCTCCTTTT 20 TCTTATCTTT TTTACCATTA TTAATAGTTA TCTGGTGTTG AACACTTTCT GTATGCCAAG TACTGGGTAA AATGTCATAA CATCCATTTC CTCATGTAAT GCTTCCGCCC ATTCTACAGG TAAGGGAAAC TGGGCTTCCC ATTGGTAGNT AAATTTTAGG TTCAGAAAGG CTTGAATTGA ATGTCAGTTC AGCCAATTTC TTAGTGGTGG AACCAAACTG AGTTCCATCC GTGAAACGGG GACARTAACA GCACCCGCTT CCCAGGGCTG GGGAAAAGTG AAGTGCAGCG GGGCAGGCAG 25 AGGACTTGAC ACAGCACTGG CCCTCAGCCA ACATCCACTA GAGGGGTGGG GTATCGCATC AGGTGGGAGA GAACTGCAAC CCTTGCAGAC AGAGGTGTGG GGCCCAGTGC AGTGATAAGA

CGGGGGTTAA CATGGGGGTG CAGGTTGTAG GATNTGGGGA CCCAAGGAGG CAGTGACGGG GCCAGGATGC CCACTCTGTA ATCACCATGC TGTGCTGGAG TTTCTGTTCC CTCAGCGCAG AGTCCTTAAA TGTGCCGCTT TTTCTNCCCT GCAGGAAGCA AGACCCGAGA AGGTGTGGTA CAAGGTGTGG CTTCAGGTAC TAGCCCAGCC CTGGCACCAG CCCTTCTCTC AMTTAGGCGG ATGATCTGGC CGGGAACCAG AGGGCGGGGG CGGGGGAGAC TCCCAAGGCT TCTGCGGGAA 5 TGCTCCGTGG GGAGGGCAGG CCCTGGGATA CTACAAGGCA GGGCATCGGT GTTTCCCCCT GGCTCCCAAA CCCCTTCCTC AACCCCCTCC CTGCTCCAGT GGCTGAAAAA ACCAAGGAAC AGGCCTCACA TCTGGGAGGA GCTGTGTTCT CTGGGGCAGG GAACATCGCA GCAGCCACAG GACTGGTGAA GAGGGAGGAA TTCCCTACTG ATCTGAAGGT AAGCGATCCT TCTGACCCGC ACATGCAGGC AAACACACAC ACACACACA ACACACCON GGCACACAAA TAAACCTGTC 10 ACCATCCCCG CCCCCTAAT CCTGCCACCA GCTTGGAACA CAAGCCACTT TGCCTCCCAT CCTGCNGGCC CGTGCTAGAC TCAGCTCAGA ATGCATCTGA ATAANGGCGT GCATGGGTGT GACGCTCCCG GTGATGGGGA CCCAGACCTG GCTGTCTGCG TGTATCCTGC TTGCCAGCGT GACCCATATG ACTTCTGGCC ACGTCTGCAT GTGTCAATGA TTGTTCATTC ATTTCTTTTC ATTCAACAAA TATCCATGCC ANANCCAGCC CTGTCCTTGA GCTTCCAGNT CCCTTTCAGC 15 CNAGGGGAGC NTGAGGGTTA TTTTTGGGGT CCCGATGCCC AGCACAGAGC CTGACACAAA GGATGAGGCA TAAGCTGGTG ANTGAGTATC CAAATGGTGG AAGTGTGGAG GNTGCCAGGC ATTGGGGGAG CGGCGTGGAG AGCCAGCTCC CCAATCCAT3 CTGCCACTTC AACTGTGATT CGGGGGAATT TCCCCCTTCA CCTCCATCCC ACTTCCAAGG CACTCCAAAT AAATAACTGA ATTAGAAATT ATCCTTGTTT TGCCAACCCA CCCTAGCCTT CCCCACTCCA ACCCACCCAA 20 AGCTTACCAC TGTGGGAATT TGGGGGGCAT CCTGGCTGTC CTCACGAGTC CTGACCTTTT CTGCCCACAG CCAGAGGAAG TGGCCCAGGA AGCTGCTGAA GAACCACTGA TTGAGCCCCT GATGGAGCCA GAAGEGGAGA GTTATGAGGA CCCACCCCAG GAGGAATATC AGGAGTATGA GCCAGAGGCG TAGGGGCCCA GGAGAGCCCC CACCAGCAGC ACAATTCTGT CCCTGTCCCT GCCCGGCCC CCAGAGCCAG GGCTGTCCTT AGACTCCTTC TCCCCAATCA CGAGATCTTC 25 CTTCCGCTCT GAGGCAACCC CCTCGGAGCC TGTGTTAGTG TCTGTCCATC TGTCTGTCCT ACCCGCCCGC GTCCAACCCC GGGGCATGGA CAGGGCCAGG GTTGCGGTCG CGGCTGGGAG

CCTCGCCCCT CCAGTGTTGC CTCCTCCCAT CCAGCGTCTG CGCG

(2) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISITCS

5 (A) LENGTH: 223 base pairs

(B) TYPE: NUCLEIC ACID

(C)STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

10 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 174P13 HUMAN GAMMA SYNULEIN GENE, 5' END

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

- AGGGAGATCC AGCTCCGTCC TGCCTGCAGC AGCACAACCC TGCACACCA CCATGGATGT

  CTTCAAGAAG GGCTTCTCCA TCGCCAAGGA GGGNGTGGTG GGTGCGGTGG AAAAGACCAA

  GCAGGGGGTG ACGGAAGCAG CTGAGAAGAC CAAGGAGGG GTCATGTATG TGGGATTACA

  TTTTTTTTT AAAGAAAGAA TAAATTAATT GTGATTAAAG TTG
- 20 (2) INFORMATION FOR SEQ ID NO:13
  - (i) SEQUENCE CHARACTERISITCS
    - (A) LENGTH: 677
    - (B) TYPENUCLEIC ACID
    - (C) STRANDEDNESS: DOUBLE
- 25 (D) TOPOLOGY: LINEAR
  - (ii) MOLECULAR TYPE: DNA (genomic)
  - (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 174P13 HUMAN GAMMA SYNULEIN GENE, 3' END

(vi)SEQUENCE DESCRIPTION: SEQ ID NO:13:

5 TTTTTNAGG GGGGAAAACA GGGAATANAA AAANANGGGG GGGGTTTTT NNGGGGGGGG
GGGGAAAANG GTTNGGGGGN NAACCNAAAN AAANNCCNAN GGGGGGGGNN ANTNAANTTT
TGGGAACCCA AAGCCCNAGG AGGATTTTN GTNAANAACG TNACCTCNAG TGGGNCGAGG
AAGACCAAGG AAANGCCCAA CNCGGTTGAN CGAGGCTGTG GTGAACANCG TNCAACNCTG
TGCCCNCCAA NANCGTGGAG GNGGCGGAGA ACATCSCGGT CACCTCCGGG GTGGTGCGCM
AGGAGGACTT GAGGCCATCT KCCCCCCMAC AGGAGGGTGT GGCATCCMAA GARAAAGAGG
AAGTGGCA3A GGAGGCCCAG AGTGGGGGAR ACTAGAGGGC TACAGGCCAG CGTGGATGAC
CTGAAGAGCG CTCCTCTGCC TTGGACACCA TCCCCTCCTA GCACAAGGAG TGCCCGCCTT
GAGTGACATG CGGCTGCCCA CGCTCCTGCC CTCGTCTTCC TGGCCACCCT TGGCCTGTCC
ACCTGTGCTG CTGCACCAAC CTCACTGCCC TCCCTCGGCC CCACCCACCC TCTGGTCCTT

- (2) INFORMATION FOR SEQ ID NO:14
- 20 (i) SEQUENCE CHAPACTERISITCS

CCACTCCAAA AAAAAAA

- (A) LENGTH: 1181 base pairs
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: DOUBLE
- (D) TOPOLOGY: LINEAR
- 25 (ii) MOLECULAR TYPE: DNA (genomic)
  - (iii) HYPOTHETICAL: NO
  - (iv) ANTI-SENSE: NO

-61-

PCT/US98/13071 WO 98/59050

## (vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene: exons 1 and 2 plus flanking intron sequences

## (viii) PCSITION IN GENOME:

5

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- (A) CHROMOSOME/SEGMENT: 4
- (B) MAP POSITION: 4q21-q22
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AATTTCAGCG ATGCGAGGGC AAAGCGCTCT CGGCGGTGCG GTGTGAGCCA CCTCCCGGCG CTGCCTGTCT CCTCCAGCAG CTCCCCAAGG GATAGGCTCT GCCCTTGGTG GTCGACCCTC AGGCCCTCGN TCTCCCAGGN CGACTCTGAC GAGGGGTAGG GGGTGGTCCC CNGGAGGACC CAGAGGAAA3 GCNGGGACAA GAAGGGAGGG GAAGGGGAAA GAGGAAGAGG CATCATCCCT AGCCCAACCG CTCCCGATCT CCACAAGAGT GCTCGTGACC CTAAACTTAA CGTGAGGCGC AAAAGCGCCC CAACCTTTTC CCGCCTTGNN CCAGGCAGGC GGCTGGAGTT GATGGCTCAC CCCGCGCCCCC CTGCCCCATC CCCATCCGAG ATAGGGACGA GGAGCACGCT GCAGGGAAAG CAGCGAGCGC CGGGAGAGGG GCGGCAGAA GCGCTGACAA ATCAGCGGTG GGGGCGGAGA GCCGAGGAGA AGGAGAAGGA GGAGGACTAG GAGGAGGAGG ACGGCGACGA CCAGAAGGGG CCCAAGAGAG GGGGCGAGCG ACCGAGCGCC GCGACGCGAA GTGAGETGCG TECGGGCTCA GCGCAGACCC CGGCCCGGCC CCTCCTGAGA GCGTCCTGGG CGCTCCCTCA CGCCTTGCCT TCAAGCCTTC TGCCTTTCCA CCCTCGTGAG CGGAGAACTG GGAGTGGCCA TTCGACGACA GGTTAGCGGG TTTGCCTCCC ACTCCCCCAG CCTCGCGTCG CCGGCTCACA GCGGCCTCCT 20 CTGGGGACAG TCCCCCCGG GTGCCCCTCC GCCCTTCCTG TGCGCTCCTT TTCCTTCTTC ENGGAGGAGT CGGAGTTGTG GAGAAGCAGA GGGACTCAGG TAAGTACCTG TGGATCTAAA CGGGNGTCTT TTGGAAATCC TGGAGAACGC CGGATGGAGA CGAATGGTCG TGGGNACCGG GAGGGGTGG TGCTGCCATG AGGACCGCTG GGCCAGGTCT CTGGGAGGTG AGTACTTGTC 25 CTTTGGGGAG CTAAGGAAAG AGACTTGACC TGGCTTCGT CCTGCTTCTG ATATTCCCTT CTCCACAAGG GCTGAGAGNT TAGGCTGCTT CTCCGGGATC C

- (2) INFORMATION FOR SEQ ID NO:15
  - (i) SEQUENCE CHARACTERISITCS
    - (A) LENGTH: 536 base pairs
    - (B) TYPE: NUCLEIC ACID
- 5 (C) STRANDEDNESS: DOUBLE
  - (D) TOPOLOGY: LINEAR
  - (ii) MOLECULAR TYPE: DNA (genomic)
  - (iii) HYPOTHETICAL: NO
  - (iv)ANTI-SENSE: NO
- 10 (vii) IMMEDIATE SOURCE:
  - (A)CLONE: human alpha symuclein gene/ exon 3 plus flanking intron sequences
    - (viii) POSITION IN GENOME:
      - (A) CHROMOSOME/SEGMENT: 4
- 15 (B) MAP POSITION: 4q21-q22
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO 15:

CTTAAAAGAG TCTCACACTT TGGAGGGTTT CTCATGATTT TTCAGTGTTT TTTGTTTATT

TTTCCCCGAA AGTTCTCATT CAAAGGGATTT TTTATGTTTT CCAGTGTGG GTAAAAGAAAT

TCATTAGCCA TGGATGTATT CATGAAAGGA CTTTCAAAGG CCAAGGAGGG AGTTGTGGCT

GCTGCTGAGA AAACCAAACA GGGTGTGGCA GAAGCAGCAG GAAAGACAAA AGAGGGTGTT

CTCTATGTAG GTAGGTAAAC CCCAAATGTC AGTTTGGTGC TTGTTCATGA GTGATGGGTT

AGGATAACAA TACTCTAAAT GCTGGTAGTT CTCTCTTG ATTCATTTT GCATCATTGC

TTGTCAAAAAA GGTGGACTGA GTCAGAGGTA TGTGTAGGTA GGTGAATGTC AACGTGTGTA

TNTGAGCTAA TAGTAAAAAT GCGACTGTTT GCTTTTCAGA TTTTTAATTT TGCCTAATAT

- 25 NTATGACTIN TTAAAATGAA TGTTTCTGTA CTACATAATT CTATNTCAGA GACAGT
  - (2) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 650 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS : DOUBLE

5 (D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

20

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(vii) IMMEDIATE SOURCE:

10 (A)CLONE: human alpha synuclein gene/ exon 4 plus flanking intron sequences

(viii) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: 4
- (B) MAP POSITION: 4q21-q22
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGCAGGTCA ACGGATCTGT CTCTAGTGCT GTACTTTTAA AGCTTCTACA GTTCTGAATT

CAAAAATTATC TTCTCACTGG GCCCCGGTGT TATCTCATTC TTTTTTCTCC TCTGTAAGTT

GACATGTGAT GTGGGAACAA AGGGGATAAA GTCATTATTT TGTGCTAAAA TCGTAATTGG

AGAGGACCTC CTGTTAGCTG GGCTTTCTTC TATNTATTGT GGTGGTTAGG AGTTCCTTCT

TCTAGTTTTA GGATATATAT ATATATTTT TCTTTCCCTG AAGATATAAT AATATATAA

CTTCTGAAGA TTGAGATTTT TAAAATTAGTT GTATTGAAAAA CTAGCTAATC AGCAATTTAA

GGCTAGCTTG AGACTTATGT CTTGAATTTG TTTTTGTAGG CTCCAAAACC AAGGAGGGAG

TGGTGCATGG TGTGGCAACA GGTAAGCTCC ATTGTGCTTA TATCAAAGAT GATATNTAAA

GTATCTAGTG ATTAGTGTGG CCCAGTATCA AGATTCCTAT TGAAATTGTA AAACAATCAC

TGAGCATCTA AGAACATATC AGTCTTATTG AAACTGAATT CTTTTAAAAG TATTTTAAA

TAGGTAAATA TTGATTATAA ATAAAAAATA TACTTGCCAA GAATAATGAG

- (2) INFORMATION FOR SEQ ID NO:17
  - (i) SEQUENCE CHARACTERISITCS
    - (A) LENGTH: 504 base pairs
    - (B) TYPE: NUCLEIC ACID
- 5 (C) STRANDEDNESS: DOUBLE
  - (D) TOPOLOGY: LINEAR
  - (ii) MOLECULAR TYPE: DNA (genomic)
  - (iii) HYPOTHETICAL: NO
  - (iv) ANTI-SENSE: NO
- 10 (vii) IMMEDIATE SOURCE:
  - (A)CLONE: human alpha synuclein gene/ exon 5 plus flanking intron sequences

#### (viii) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: 4
- 15 (B) MAP POSITION: 4q21-q22
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATATCTTAGC CAAGATTCAA TGTTTGGTTG AACCACACTC ACTTGACATC TTGGTGGCTT

TTGTTTCTTC TGACCACTCA GTTATCTATG GCATGTGTAG ATACAGGTGT ATGGAANCGA

TGGCTAGTGG AAGTGGAATG ATTTTAAGTC ACTGTTATTC TACCACCCTT TAATCTGTTG

TTGCTCTTTA TTTGTACCAG TGGCTGAGAA GACCAAAGAG CAAGTGACAA ATGTTGGAGG

AGCAGTGGTG ACGGGTGTGA CAGCAGTAGC CCAGAAGACA GTGGAGGGAG CAGGGAGCAT

TGCAGCAGCC ACTGGCTTTG TCAAAAAGGA CCAGTTGGGC AAGGTATGGC TGTGTACGTT

TTGTGTTACA TTTATAAGCT GGTGAGATTA CGGTTCATTT TCATGTGAAG CCTGGAGGCA

GGAGCAAGAT ACTTACTGTG GGGAACGGCT ACCTGACCCT CCCCTTGTGA AAAAGTGCTA

- 25 CCTTTATATT GGTCTTGCTT GTTT
  - (2) INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISITCS

(A) LENGTH: 727 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

5 (D) TCPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(vii) IMMEDIATE SOURCE:

10 (A)CLONE: human alpha symuclein gene exons 1 and 2 plus flanking intron sequences

(viii) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: 4
- (B) MAP POSITION: 4q21-q22
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TAGCTTAATA TACNTACTAC TTGACCCTTT ACAGGAAAAG CTTTACTAAC CCCTGCATTA
GAGAATATAT TTTTTTGCAA AAACATTGAT TGTAAATTTT AGTGTAAAGT GGGGAGCCAT
TTCCTATCTC ATTGGCTGTC CAGTGCTGAT GCGTAATTGA AACTTATACT AACAGTGTGT

#### GCTGTCT

- (2) INFORMATION FOR SEQ ID NO:19
  - (i) SEQUENCE CHARACTERISITCS
- 5 (A) LENGTH: 1596 base pairs
  - (B) TYPE: NUCLEIC ACID
  - (C)STRANDEDNESS:DOUBLE
  - (D) TOPOLOGY: LINEAR
  - (ii) MOLECULAR TYPE: DNA (genomic)
- 10 (iii) HYPOTHETICAL: NO
  - (iv) ANTI-SENSE: NO
  - (vii) IMMEDIATE SOURCE:
  - (A)CLONE: human alpha synuclein gene/ exon 7 plus flanking intron sequences
- 15 (viii) POSITION IN GENOME:
  - (A) CHROMOSOME/SEGMENT: 4
  - (B) MAP POSITION: 4q21-q22
  - (x1) SEQUENCE DESCRIPTION: SEQ ID NO:19:
- TTTTGATTT TCTAATATTA GGAAGGGTAT CAAGACTACG AACCTGAAGC CTAAGAAATA

  20 TCTTTGCTCC CAGTTCTTG AGATCTGCTG ACAGATGTTC CATCCTGTAC AAGTGCTCAG

  TTCCAATGTG CCCAGTCATG ACATTTCTCA AAGTTTTTAC AGTGTATCTC GAAGTCTTCC

  ATCAGCAGTG ATTGAAGCAT CTGTACCTGC CCCCACTCAG CATTTCGGTG CTTCCCTTTC

  ACTGAAGTGA ATACATGGTA GCAGGGTCTT TGTGTGCTGT GGATTTTGTG GCTTCAATCT

  ACGATGTTAA AACAAATTAA AAACACCTAA GTGACTACCA CTTATTTCTA AATCCTCACT
- 25 ATTTTTTGT TGCTGTTGTT CAGAAGTTGT TAGTGATTTG CTATCATATA TTATNAGATT
  - TTTAGGTGTC TTTTAATGAT ACTGTCTAAG AATAATGACG TATTGTGAAA TTTGTTAATA
  - TATATNATAC TTAAAAATAT GTGAGCATGA AACTATGCAC CTATAATACT AAATATGAAA

TTTTACCATT TTGCGATGTG TTTTATTCAC TTGTGTTTGT ATATNAATGG TGAGAATTAA AATAAAAGGT TATCTCATTG CAAAAATATT TTATTTTTAT GCCATCTCAC TTTAATAATA AAAATCATGC TTATAAGCAA CATGAATTAA GAACTGACAC AAAGGACAAA AATATAAAGT TATTAATAGC CATTTGAAGA AGGAGGAATT TTAGAAGAGG TAGAGAAAAT GGAACATTAA CCCTACACTC GGAATTCCCT GAAGCAACAC TGCCAGAAGT GTGTTTTGGT ATGCACTGGT TCCTTAAGTG GCTGTGATTA ATTATTGAAA GTGGGGTGTT GAAGACCCCA ACTACTATTG TAGAGTGGTC TATTTCTCCC TTCAATCCTG TCAATGTTTG CTTTACGTAT TTTGGGGAAC TGTTGTTTGA TGTGTATGTG TTTATAATTG TTATACATTT TTAATTGAGC CTTTTATTAA CATATATTGT TATTTTTGTC TCGAAATAAT TTTTTAGTTA AAATCTATTT TGTCTGATAT TGGTGTGAAT GCTGTACCTT TCTGACAATA AATAATATNC GACCATGAAT AAAAAAAAAA AAAAAGTGGG TTCCCGGGAA CTAAGCAGTG TAGAAGATGA TTTTGACTAC ACCCTCCTTA GAGAGCCATA AGACACATTA GCACATATTA GCACATTCAA GGCTCTGAGA GAATGTGGTT AACTTTGTTT AACTCAGCAT TCCTCACTTT TTTTTTTTAA TCATCAGAAA TTCTCTCTCT AACATCGTTG GGAACTACCA GAGTCACCTT AAAGGGAGNA TCAATTCTCT AGGACTGGAT AAAAATTTCA TGGGCCTCCT TTAAAATGTT GCCCAAATAT ATGGAATTCT AGGGGTTTTT CCNTAGGGGG AAGGGTTTTT TCTCTTTTCN GGGGAGGATC CTTTTAACNC CCCNGGGGGG NGCCCGGAAA ATAAACTTGG NGGGGGGGNA AAACTT

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#### WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising a nucleotide sequence encoding a mutated human synuclein protein or homologue thereof.

- 5 2. The isolated nucleic acid of claim 1 wherein said mutated synuclein protein is selected from the group consisting of alpha, beta and gamma synuclein proteins.
- 3. The isolated nucleic acid of claim 2 wherein said mutated10 synuclein protein is the alpha synuclein protein.
  - 4. The isolated nucleic acid of claim 3 wherein said nucleotide sequence contains at least one mutation at base pair position 209.
- 15 5. The isolated nucleic acid of claim 4 wherein said mutation at position 209 is a change from guanine to adenine.
  - 6. The isolated nucleic acid of claim 5 having the sequence given in SEQ ID NO. 1.
  - 7. An oligonucleotide complementary to a portion of the synuclein gene, wherein said portion comprises a mutation associated with predisposition to Parkinson's Disease.
- 25 8. The oligonucleotide of claim 7 wherein said mutation is at base pair position 209 in the symnuclein gene.

9. The oligonucleotide of claim 8 wherein said mutation is a change from guanine to adenine.

10. A vector comprising the isolated nucleic acid of claim 1.

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- 11. A host cell comprising the vector of claim 10.
- 12. A method of affecting characteristics of Parkinson's Disease, comprising of expressing nucleic acids which are implicated in disease development in cultured cells through the use of expression vectors.
  - 13. The method of claim 12 wherein the said nucleic acid is selected from the group consisting of alpha, beta, and gamma synuclein genes.
- 14. The method of claim 13 wherein the said nucleic acid encodes the mutated alpha synuclein protein.
  - 15. The method in claim 14 wherein the said mutated alpha synuclein protein contains at least one mutation at base pair 209.
- 20 16. The method of claim 15 wherein said mutation at position 209 is a change from guanine to adenine.
  - 17. An isolated human synuclein protein or peptide containing at least one mutation.

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18. The isolated human synuclein protein or peptide of claim 17 wherein said protein or peptide is selected from the group consisting of

the human alpha, beta and gamma synuclein proteins or fragments thereof.

19. The isolated human synuclein protein or peptide of claim 18 having the sequence given in SEQ ID NO 5.

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- 20. The isolated human synuclein protein or peptide of claim 19 wherein said protein or peptide is the alpha synuclein gene or a fragment thereof.
- 10 21. The isolated protein or peptide of claim 20, wherein said mutation is at amino acid position 53.
  - 22. The isolated protein or peptide of claim 21, wherein said mutation is an alanine to threonine substitution.

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- 23. An antibody specific for the protein or peptide of claim 17.
- 24. A method of detecting subjects at increased risk for Parkinson's Disease, comprising:
- obtaining a sample comprising nucleic acids, proteins or tissues from the subjects, and

detecting in the nucleic acids, proteins or tissues the presence of a mutation which is associated with Parkinson's disease,

thereby identifying subjects at increased risk for the disease.

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25. The method of claim 24 wherein said mutation is located on human chromosome four.

26. The method of claim 25 wherein said mutation is located in the alpha symuclein gene.

- 27. The method of claim 26 wherein said mutation causes an amino acid substitution at position 53.
  - 28. The method of claim 27 wherein said mutation causes an alanine to threonine substitution at position 53.
- 29. The method of claim 24 wherein said detecting step comprises combining a nucleotide probe which selectively hybridizes to a nucleic acid containing said mutation, and detecting the presence of hybridization.
- 30. The method of claim 29 wherein said nucleotide probe is an oligonucleotide complementary to a portion of the synuclein gene, wherein said portion comprises a mutation associated with predisposition to Parkinson's Disease.
- 20 31. The method of claim 30 wherin the mutation of said oligonucleotide is at base pair position 209 in the alpha synuclein gene.
  - 32. The method of claim 31 wherein the mutation of said oligonucleotide is a change from guarine to adenine.

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33. The method of claim 24 wherein said detecting step comprises amplifying a nucleic acid product comprising said mutation, and detecting

the presence of said mutation in the amplified product.

34. The method of claim 33 wherein said detecting step comprises selectively amplifying a nucleic acid product comprising said mutation, and detecting the presence of amplification.

35. The method of claim 34 wherein said amplifying step comprises at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids.

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- 36. The method of claim 35 wherein said amplifying step uses two oligonucleotides.
- 37. The method of claim 36 wherein said two oligonucleotides have the sequences of SEQ ID NOs 2 and 3.
  - 38. The method of claim 24 wherein said detecting step comprises detecting the presence or absence of a restriction endonuclease site as detected by enzymatic digest of said sample of nucleic acids.

- 39. The method of claim 38 wherein said restriction endonuclease site is recognized by Tsp451.
- 40. The method of claim 24 wherein said detecting step comprises chain termination with a labeled dideoxynucleotide.
  - 41. An oligonucleotide complementary to a nucleic acid sequence

which flanks a mutation in the symuclein gene that is associated with predisposition to Parkinson's disease, wherein said oligonucleotide may be used in diagnostic screens in the amplification of a nucleic acid sequence comprising said mutation.

- \$42\$. The cligonucleotide of claim 41 having the sequence of SEQ ID NO 2.
- \$43.\$ The cligonucleotide of claim 41 having the sequence of SEQ ID  $$10^{\circ}$$  NO 3.
  - 44. The method of claim 24 wherein said detection step comprises identification of said mutations with an antibody.
- 15 45. The method of claim 44 wherein said antibody is directed against an isolated human synuclein protein or peptide containing at least one mutation.
- 46. The method of claim 45 wherein said isolated human synuclein protein or peptide is selected from a group consisting of the human alpha, beta, and gamma synuclein proteins or fragments thereof.
  - 47. The method of claim 46 wherein said isolated human symuclein protein or peptide has the mutated sequence given in SEQ ID NO 5.
- 25
- 48. The method of claim 47 wherein said mutation is at amino acid position 53.

49. The method of claim 48 wherein said mutation is an alanine to threonine substition

50. A diagnostic kit comprising the oligonucleotide of claim 41.

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- 51. A diagnostic kit comprising the oligonucleotide of claim 42.
- 52. A diagnostic kit comprising the oligonucleotide of claim 43.
- 10 53. A diagnostic kit comprising the oligonucleotide of claim 7.
  - 54. A diagnostic kit comprising the oligonucleotide of claim 8.
  - 55. A diagnostic kit comprising the oligonucleotide of claim 9.

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- 56. A diagnostic kit comprising the antibody of claim 23.
- 57. An isolated nucleic acid comprising a mutation in a human synuclein gene or homologue thereof.

- 58. The isolated nucleic acid of claim 57 wherein said synuclein gene is the alpha synuclein gene.
- 59. The isolated nucleic acid of claim 58 wherein said mutation occurs at base pair position 209.
  - 60. The isolated nucleic acid of claim 59 wherein said mutation is

a change from guanine to adenine.

61. The isolated nucleic acid of claim 60 having the sequence given in SEO ID NO 1.

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- 62. A transgenic animal which expresses a mutated synuclein protein, wherein said animal may be used as an animal model for Parkinson's disease.
- 10 63. The transgenic animal of claim 62, wherein said mutated synuclein protein is an alpha synuclein protein.
  - 64. A method of screening a compound for the ability to reverse the self-aggregation of synuclein proteins, comprising exposing an aggregate of synuclein proteins to a test compound and observing whether or not the aggregate is dissolved.
  - 65. The method of claim 64 wherein said test compound is a synuclein peptide.

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- 66. The method of claim 65 wherein said peptide comprises a mutation.
- 67. The method of claim 64 wherein said test compound is an 25 antibody.
  - 68. The method of claim 64, wherein said observing step comprises

Congo red staining, electron microscopy or CD spectrometry.

69. The method of claim 64 wherein said protein aggregate is located within an animal.

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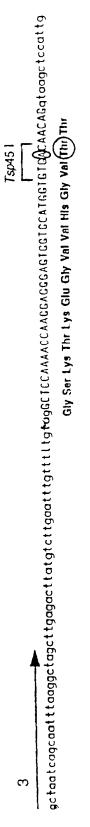
10

- 70. A method of screening a compound for the ability to inhibit the self-aggregation of synuclein proteins, comprising exposing a population of synuclein proteins to a test compound under conditions which promote self-aggregation in the absence of said compound and observing whether or not self-aggregation of said proteins is inhibited.
- 71. The method of claim 70 wherein said test compound is a synuclein peptide.
- The method of claim 71 wherein said peptide comprises a mutation.
  - 73. The method of claim 70 wherein said test compound is an antibody.

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74. The invention substantially as disclosed and described.

Figure 1



ty etta la tecada gata ta taga que te taga que taga que te aga de tecada de acta que atta que taga de tetada que esta que ta taga de tetada que esta que ta taga que taga que ta taga que ta taga que ta taga que ta taga que taga que ta taga que taga que



FIG. 2

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Homo sapiens Rattus norvegicus Bos taurus Serinus canaria Torpedo californica	Homo sapiens Rattus norvegicus Bos taurus Serinus canaria Torpedo californica	Homo sapiens Rattus norvegicus Bos taurus Serinus canaria Torpedo californica	Homo sapiens Rattus norvegkus Bos taurus Serinus canarla Torpedo cattomica
10  I  MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLY MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLY MDVFMKGLSMAKEGVVAAAEKTKQGVTEAAEKT MDVFMKGLSMAKEGVVAAAEKTKQGVTEAAEKT MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLY MDVLKKGFSFAKEGVVAAAEKTKQGVQDAAEKTKQGVQDAAEKTKEGVMY	KTKEGV KTKEGV KTKEGV RTKEGV	110  1	130  1
	40 40 40 51	90 79 90 101	

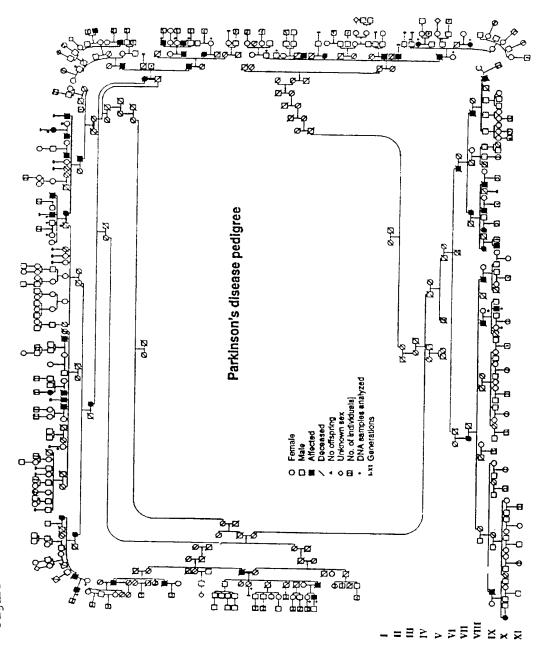
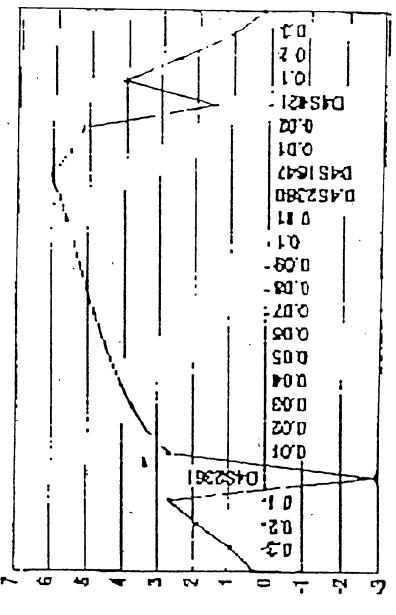


Figure 5





rop score

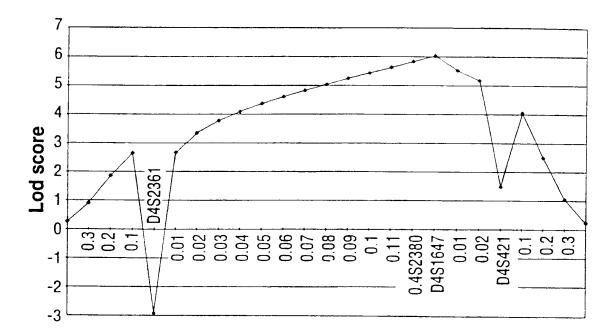


FIG. 6

Figure 7/1

clone	5'	3 '	gene
109979	T84229	T88834	alpha
111088	T83410	1000	aloha
	TB3411	T81593	aloha
111090	R11619	(R19409)	alpha
130048		R32856	alpha
135534	R31354	R67383	alpha
141246	R66663	R77746	alpha
145594	R78091		
171906	H19290	H19291	beta
172284	H19556	H19474	beta
172749		H19685	beta
175546		H41126	beta
193174	H47503	H47504	alpha
210768	H66914	H66869	alpha
213616	H70324	H70325	aloha
236027	H62070	·	alpha
248153	N53829	N73325	alpha
24991	(T80528)	R39000	aloha
26298	R13508	(R20629)	alpha
265817	N28661	N21457	alpha
266628		N22757	alpha
27342		R37173	alpha
280344	(N50305)	N47094	alpha
290894		N72005	aloha
294142		N68597	alpha
307787	W21278	<u> </u>	alpha
340635	W56712	W56757	alpha
340683	W55988	W56276	alpha
346647	W94390	W74638	alpha
346796	W79585	W79784	alpha
359349	AA010546	AA010547	alpha
364632	AA022809	AA022690	alpha
39915		R50455	beta
40764	R56327	R56245	alpha
45086	H08908	H08824	aipha
48607	H10267	H10213	alpha
49811	H29080	H2B976	alpha
50202		H17962	beta
50470		H16811	beta
66473	R16018	R16119	alpha
687794	AA258686	AA258608	alpha
69907	T48654	T48655	alpha
72391	AA394097	AA293803	gamma
739009	AA421586		beta
739014	(AA42185)	AA421567	beta
771303		AA443638	gamma
2-4		L36675	alpha
2-5		L36874	alpha
c-01f06		F01363	alpha
c-1rb08	F03254	F06981	alpha
c-2td12	F08836	F11169	alpha
c-28f08	F03751	F07521	alpha
cDNA	S69965		beta
EST01420	M79265		gamma
(HRBAA27)			
EST19193	AA317129		beta
EST22040	AA319774		elpha

Figure 7/2

EST26845	T28079		beta
EST31489	AA328063		aipha
EST68G11	W22518		gamma
F1-625D	R29481		alpha
GEN-129D09	D61090		beta
hbc590	T11070		alpha
HIBBA65	T08213	T08212	alpha
	HR70E3R	HR70E3F	alpha
HSNACP0		U46896- 46901	alpha
10000		40901	<del></del>
KK1311	N83633		alpha
		D318839	alpha
		L08850	alpha
	T28735		alpha
	Z20502		alpha

Figure 8

10	20	30	40	50	60	70
CCGCCGCAGCCGCCG AGGGGCCCGGGAXAA TCCGCGGCCCTGGAG CAGAAGGGCXCCGCG GGGGCCAGTGCACCG	AAAXCGAGCAG GTTCGCACTGG GTCTCGGGAGT	GCCCCGGCCC TGGCCCTTCC GGAGTGGGGT AGGGGGGCAT	CGCATCCGGT CGCGTCCCCA GAGATGGGGG XTGCGTCCCG	TTGGAAGGGG GGGTTTCAAG GAAAGCGGGA CGGGAGGGG	GCTGCAAGT GGACGCTAG AGGGGGCTCA CAGGGGGAAG	GAXTX 140 GGGTC 210 AGTGC 280
360	370	380	390	400	410	420
TGTCCATGGCCAAGG GAAGACCAAGGAGGG TCCCCCTACAGTGTG AGATGGGGCXAGGTC AATGGGGACACGGGG	AGGGCGTTGTG CGTCCTCTACG GAGCTGGGGCC AXCAXGGGTCA CGGGCTGATGA 720	GCAGCCGCGG TCGGTGGGCX GGGTCCCGGG TAAGGGACAT GGTGGGGGGC 730	AGAAAACCAA GGGGGCXGGG GAGGGGGTT ACCCAXCCCA TCCAXCTGAA 740	GCAGGGGGTC TTTCTGGGGC CTGGGCAAGA TAGAAXCCTG AGGCCAGGGA 750	ACCGAGGCG TGCAGGGCT TAATATXAX GGTCTGTAT CCAXTGCAX 760	GCGGA 420 GGGGG 490 TCAGC 560 CCGGA 630
AAAXCACACAXCCTC						TITCI 770
GTATGCCAAGTACTO TAAGGGAAACTGGGC AGCCAATTTCTTAGT CCCAGGGCTGGGGAA	GGTAAAATGTC TTCCCATTGGT GGTGGAACCAA	ATAACATCCA AGXTAAATTT ACTGAGTTCC	TTTCCTCATG TAGGTTCAGA ATCCGTGAAA	TAATGCTTCC AAGGCTTGAA CGGGGGACAAT	GCCCATTCT. TTGAATGTC. AACAGCACC	ACAGG 840 AGTTC 910 CGCTT 980 AGCCA 1050
<u></u>	<del></del>	بليستليب		بالمسلمين	البيليين	1120
ACATCCACTAGAGGG GGCCCAGTGCAGTGA CAGTGACGGGGCCAG AGTCCTTAAATGTGG CITCAGGTACTAGCG	TAAGACGGGGG GTAACCCACTC TTTTTCTX	TTAACATGGG TGTAATCACC CCCTGCAGGA	GGTGCAGGTT ATGCTGTGCT AGCAAGACCC	GTAGGATXTG GGAGTTTCTG GAGAAGGTGT	AADDDADDDI DAD1DDDTTI DDAADATDDI	GGAGG 1190 CGCAG 1260 TGTGG 1330
1410	1420	1430	1440	1450	1460	1470
AGGGCGGGCCGGGCAGCAGCAGCAGCAGCAAAAAACCAAGCAGC	AGAGACTCCCAA ATCGGTGTTTCC AGGAACAGGCCT AGGAGAGAGGGA	GGCTTCTGCG CCCTGGCTCC CACATCTGGG GGAATTCCCT	GGAATGCTCC CAAACCCCTT AGGAGCTGTG ACTGATCTGA	GTGGGGAGGG CCTCAACCCC TTCTCTGGG AGGTAAGCGA	CAGGCCCTG CTCCCTGCT CAGGGAACA CTCCTTCTGA	CCAGT 1540 TCGCA 1610 CCCGC 1680
1760	1770	1780	1790	1800	1810	1820
CCCCCCTAATCCTGC TCAGCTCAGAATGCA GCTGTCTGCGTGTA TTGTTCATTCATTTC CCCTTTCAGCCXAGC	CACCAGCTTGC ATCTGAATAAXC CCTGCTTGCCA	AACACAAGCC GCGTGCATGG GCGTGACCCA ACAAATATCCA	ACTTTGCCTC GTGTGACGCT TATGACTTCT TGCCAXAXCC	CCATCCTGCX CCCGGTGATG GGCCACGTCT AGCCCTGTCC	GGCCCGTGC GGGACCCAG GCATGTGTC TTGAGCTTC	TAGAC 1820 ACCTG 1890 AATGA 1960 CAGXT 2030
GGATGAGGCATAAG		TATCCANATC				
CGGCGTGGAGAGCC. CCTCCATCCACTT CCCTAGCCTTCCCC. CTCACGAGTCCTGA	AGCTCCCAAT( CCAAGGCACTC( ACTCCAACCCA(	CATGCTGCCA CAAATAAATAA CCCAAAGCTTA CACAGCCAGAG	CTTCAACTGT CTGAATTAGA CCACTGTGGG GAAGTGGCCC	GATTCGCGG AATTATCCTI GQQQQTTTAA	GTTTTCCCC GTTTTTCCA GCATCCTGG	CTTCA 2240 ACCCA 2310 CTGTC 2380
2460	2470	2480 	2490	2500 	2510	2520
TTGAGCCCCTGATG GCCAGAGGCGTAGG CCAGAGCCAGGGCT CCTCGGAGCCTGTG CAGGGCCAGGGTTG	GAGCCAGAAGG GGCCCAGAGA GTCCTTAGACT TTAGTGTCTGT CGGTCGCGGCT	GGAGAGTTATG GCCCCCACCAG CCTTCTCCCA CCATCTGTCTG GGGAGCCTCGC	AGGACCCACO CAGCACAATT ATCACGAGAT TCCTACCCGC CCCTCCAGTC	CCAGAGAGAA CCTGTCCTGT CCTCCTTCCC AACCTCCCCCCCCCC	ATATCAGGAG CCCCTGCCCC CCTCTGAGGC ACCCCGGGGC CCCATCCAGC	TATGA 2520 GCCCC 2590 AACCC 2660 ATGGA 2730 GICTG 2800
2810	2820 111	2830 	2840	2850 	2860	2870
CGCG 2804						

Figure 9

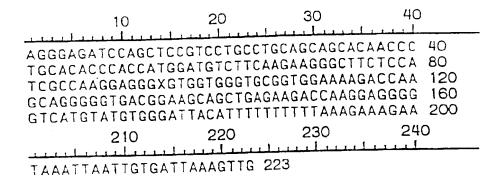
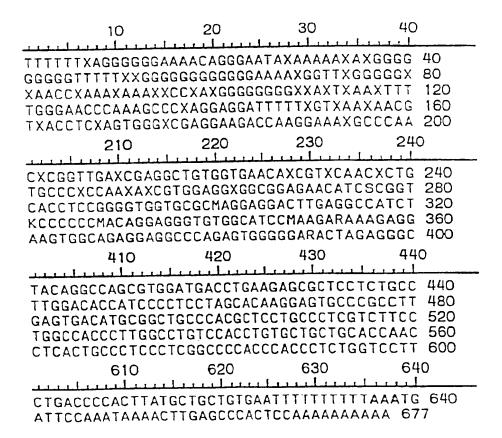


Figure 10



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Figure 11/1 alpha-SYN exons 1-2

10	20	30	40
AATTTCAGCGATGCG GTGTGAGCCACCTCC CTCCCCAAGGGATAG AGGCCCTCGNTCTCC GGGTGGTCCCCNGGA	AGGGCAAAGC CGGCGCTGCC GCTCTGCCCT CAGGNCGACT	GCTCTCGGC TGTCTCCTC TGGTGGTCG CTGACGAGG	GGTGCG 40 CAGCAG 80 ACCCTC 120 GGTAGG 160
210	220	230 	240
GAAGGGAGGGAAGG AGCCCAACCGCTCCC CTAAACTTAACGTGA CCGCCTTGNNCCAGG CCCGCGCCCCCTGC	AGGAAAGAGA AGACTCTAGO AAAAAGGCGCAAAA AGGCGGCTG	AAGAGGCATO AAGAGTGCTE GCGCCCCAAC GGAGTTGATO	CTTTTC 320 GGCTCAC 360 GGGACGA 400
410	420 11	430	440
GGAGCACGCTGCAG GCGGGCAGAAGCGC GCCGAGGAGAAGGA ACGGCGACGACCAG ACCGAGCGCCGCGA	GGAAAGCAGC( TGACAAATCA) GAAGGAGGAG AAGGGGCCCA	GAGCGCCGG( GCGGTGGGG( GACTAGGAG( AGAGAGGGG	GAGAGGG 440 GCGGAGA 480 GAGGAGG 520 GCGAGCG 560
610	620	630	640
GCGCAGACCCCGGC CGCTCCCTCACGCC CCCTCGTGAGCGGA GGTTAGCGGGTTTC CCGGCTCACAGCGC	CCGGCCCCTC TTGCCTTCAA GAACTGGGAG GCCTCCACTC	CTGAGAGCG CCCTTCTGC CTGGCCATTC CCCCAGCCT	TCCTGGG 640 CTTTCCA 680 GACGACA 720 CGCGTCG 760
810	ببيليبين	ببلبين	
GTGCCCCTCCGCC TTTCCTATTAAAT TTTTAAAAAAAGA GAGAAGCAGAGGG CGGGNGTCTTTGG	ATTATTTGGG/ GAGAGGCGNG( ACTCAGGTAA(	AATTGTTTAA GAGGAGTCG0 GTACCTGTG0 GAACGCCGGA	AATTTTTT 880 AAGTTGTG 920 BATCTAAA 960 ATGGAGAC 1000
1010	1020	1 <b>0</b> 30	1040
GAATGGTCGTGGG GGACCGCTGGGCC TTTGGGGAGCCTA CCTGCTTCTGATA TAGGCTGCTTCTC	NACCGGGAGG AGGTCTCTGG AGGAAAGAA ATTCCCTTCTC	GGGTGGTGC GAGGTGAGT, CTTGACCIG CACAAGGGC	TGCCATGA 1040 ACTTGTCC 1080 GCTTTCGT 1120

10	20	30	40
<u> </u>	بلينديلينين	<u> </u>	<u> </u>
CTTAAAAGAGTCTCA	CACTTTGGAGG	GTTTCTCA	TGATTT 40
TTCAGTGTTTTTTGT	TTATTTTCCC	CGAAAGTT	CTCATT 80
CAAAGTGTATTTAT			
TCATTAGCCATGGAT	GTATTCATGAA	AGGACTTT	CAAAGG 160
CCAAGGAGGGAGTTG	TGGCTGCTGCT	GAGAAAAC	CAAACA 200
210	220	230	240
<del></del>	<u> </u>		<u> </u>
GGGTGTGGCAGAAGC	AGCAGGAAAGA	CAAAAGAG	GGTGTT 240
CTCTATGTAGGTAGG	TAAACCCCAAA	TGTCAGTT	TGGTGC 280
TTGTTCATGAGTGAT	GGGTTAGGATA	ACAATACTO	CTAAAT 320
GCTGGTAGTTCTCTC	TCTTGATTCAT	TTTTGCATO	CATIGO 360
TTGTCAAAAAGGTGG	ACTGAGTCAGA	GGTATGTG	TAGGTA 400
410	420	430	440
<del></del>		<u></u>	1 1 1 1 1
GGTGAATGTGAACGT			
GCGACTGTTTGCTTT	TCAGATTTTTA	ATTTTGCCT	TAATAT 480
NTATGACTINTTAAA	ATGAATGTTTC	TGTACTACA	TAATT 520
CTATNTCAGAGACAG	T 536		

10 20	30	40
<del></del>	<del>- 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1</del>	<u> </u>
CTGCAGGTCAACGGATCTGTCTC	TAGTGCTGTACT	TTTAA 40
AGCTTCTACAGTTCTGAATTCAA	AATTATCTTCTC	ACTGG 80
GCCCCGGTGTTATCTCATTCTTT	TTTCTCCTCTGT	AAGTT 120
GACATGTGATGTGGGAACAAAGG	GGATAAAGTCAT	
TGTGCTAAAATCGTAATTGGAGAG	GGACCTCCTGTT	AGCTG 200
210 220	230	240
<del></del>	<del> </del>	4-4-4-1
GGCTTTCTTCTATNTATTGTGGT		
TCTAGTTTTAGGATATATATATA	TATTTTTTCTT	TCCCT 280
GAAGATATAATAATATATACT	TCTGAAGATTGA	GATTT 320
TTAAATTAGTTGTATTGAAAACT	AGCTAATCAGCA	ATTTA 360
AGGCTAGCTTGAGACTTATGTCT	TGAATTTGTTTT	TGTAG 400
410 420	430	440
<del></del>	<del> </del>	
GCTCCAAAACCAAGGAGGGAGTG	GTGCATGGTGTG	GCAAC 440
AGGTAAGCTCCATTGTGCTTATA	TCAAAGATGATA	
AGTATCTAGTGATTAGTGTGGCC	CAGTATCAAGAT	TCCTA 520
TGAAATTGTAAAACAATCACTGA	GCATCTAAGAAC	ATATC 560
AGTCTTATTGAAACTGAATTCTT	TATAAAGTATTT	TTAAA 600
610 620	630	640
610 620	630	640
	630	640

	10	20	30 :	40
ACTTGAC. GTTATCT. TGGCTAG	ATCTTGGTGG ATGGCATGTG TGGAAGTGGA	CAATGTTTGGT CTTTTGTTTCT TAGATACAGGT ATGATTTTAAG TTGTTGCTCTT	TCTGACCACT GTATGGAANO TCACTGTTAT	CA 80 CGA 120 TC 160
<u></u>	210	220	<del></del>	240
AGCAGTG GTGGAGG TCAAAAA	GTGACGGGTG GAGCAGGGAG GGACCAGTTG GCATTTATAA	GAGCAAGTGAC TGACAGCAGTA CATTGCAGCAG GGCAAGGTATG GCTGGTGAGAT	GCCCAGAAGA CCACTGGCT1 GCTGTGTAC0 TACGGTTCA1	ACA 280 FTG 320 GTT 360 FTT 400
	410	420 	430 	440
GGGAACG		GCAGGAGCAAG CCTCCCCTTGT		

Figure 11/5

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10	20	30	40	
AAAAGTTTACATACTT CAATGTTTCCCCGGAG TAGTAATATTAAGGTG ACATCCCTATATGTAA TTTTTAAAAGTGAAAA	TGCCATTT	TCAAGATCCG CAAAACATGG TCATCATGTT 230	TGGCCA 120 TTCTGA 160	_
GTGCTTCTTACTTTACACACACACACATTCTCTAACACACAC	AATATTAGA GGAAGATAT ATGCCTTCT	ATGAAGAAGA GCCTGTGGAT GAGGTAGGAG TCCAAAAACT	TCCAAG 320 GTCATT 360	
GCTCTCTACATGCTC GAATAGTTTTTACAT AGGAGGAGGAAGATC GAAATCATATGTAGT TTGACCCTTTACAGC	TTTTTAAAGAAGAGAAGAAGACACACATAG TCCACATAG TAAAAAGCTT	GAAGAAAGGA CTTAATATAC	TGTAAAA 520 NTACTAC 560	
GAGAATATATTTT AGTGTAAAGTGGGG CAGTGCTGATGCGT GCTGTCT 727	$\Lambda$ CCC $\Lambda$ 1 $\pm$ 11	ILIAIUIUALI	adcidio oco	

10	20	30	40
TTTTGATTTTTCTAATA AACCTGAAGCCTAAGAA AGATCTGCTGACAGATG	ATATCTTTGC	TCCCAGTTTC	TTG 80
TTCCAATGTGCCCAGTC AGTGTATCTCGAAGTCT	ATGACATTTC TCCATCAGCA	TCAAAGTTTT GTGATTGAAG	TAC 160 CAT 200
210	220 	230 	240
CTGTACCTGCCCCACT ACTGAAGTGAATACATG GGATTTTGTGGCTTCAA AAACACCTAAGTGACTA ATTTTTTTGTTGCTGTT	GTAGCAGGGT( TCTACGATGT CCACTTATTT(	CTTTGTGTGC FAAAACAAAT CTAAATCCTC	TGT 280 TAA 320 ACT 360
<del></del>	بيلينيلي	<del> </del>	<u> </u>
CTATCATATATTATNAG. ACTGTCTAAGAATAATG. TATATNATACTTAAAAA CTATAATACTAAATATG TITTATTCACTTGTGTT	ACGTATTGTG/ TATGTGAGCA AAATTTTACC/	AAATTTGTTA/ TGAAACTATG( ATTTTGCGAT(	ATA 480 CAC 520 GTG 560
610	620	630	640
AATAAAACGTTATCTCA CCCATCTCACTTTAATA CATGAATTAAGAACTGA TATTAATAGCCATTTGA TAGAGAAAATGGAACAT	TTGCAAAAATA ATAAAAATCA CACAAAGGACA AGAAGGAGGAA	ATTTTATTTT TGCTTATAAG AAAAATATAA ATTTTAGAAG	TAT 640 CAA 680 AGT 720 AGG 760
810	820	830	840
GAAGCAACACTGCCAGA TCCTTAAGTGGCTGTGA GAAGACCCCAACTACTA TTCAATCCTGTCAATGT TGTTGTTTGATGTGTAT	AGTGTGTTTTC TTAATTATTG, TTGTAGAGTGC TTGCTTTACG GTGTTTATAA	GGTATGCACTO AAAGTGGGGTO GTCTATTTCTO TATTTTGGGG TTGTTATACA	GTT 880 CCC 920 AAC 960 TTT 1000
1010	1020	1030	1040
TTAATTGAGCCTTTTAT TCGAAATAATTTTTTAG TGGTGTGAATGCTGTAC GACCATGAATAAAAAA CTAAGCAGTGTAGAAGA	TAACATATAT TTAAAATCTA CTTTCTGACA AAAAAAAAGT	TGTTATTTTT TTTTGTCTGA ATAAATAATA GGGTTCCCGG	TAT 1080 TNC 1120 GAA 1160

1210	1220	1230	1240
GAGAGCCATAAGAC GGCTCTGAGAGAAT TCCTCACTTTTTTT CTCTCTCTTTTTCT TTTTACAGGAAATG	ACATTAGCACA GTGGTTAACTT TTTTAATCATC CTCGCTCTCTT	TATTAGCACA TGTTTAACTO AGAAATTCTC TTTTTTTTT	ATTCAA 1240 CAGCAT 1280 CTCTCT 1320 FTTTTT 1360
1410	1420		1440

Inter onal Application No. PCT/US 98/13071

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C12N15/12 C07K14/47

C12Q1/68

G01N33/68

C12N15/11 A01K67/027 C07K16/18

A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

#### **B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C12N C07K A61K C12Q G01N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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	XP002083889	
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	27 February 1996	62-73
	see the whole document	

X Further documents are listed in the continuation of box C	X Patent family members are listed in annex
"A" document defining the general state of the art which is not considered to be of particular relevance."  "E" eadlier document but published on or after the international filing date.  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another.	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention.  "X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone.  "Y" document of particular relevance, the claimed invention.
citation or other special reason (as specified)  "O" document referring to an oral disclosure, use, exhibition or other means  "P" document published prior to the international filing date but later than the priority date claimed	cannot be considered to involve an inventive step when the document is combined with one or more other such documents such combination being obvious to a person skilled in the art.  3. document member of the same patent family
Date of the actual completion of the international search  10 November 1998	Date of mailing of the international search report 27/11/1998
Name and mailing address of the ISA  European Patent Office P.B. 5818 Patentiaan 2  NL - 2280 HV Rijswijk  Tel. (+31.70) 340-2040, Tx. 31.651 epo.nl  Fax: (+31-70) 340-3016	Authorized officer  Mand 1 , B

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